VARIANTS OF PROTEIN KINASES

FIELD OF THE INVENTION

The present invention concerns novel nucleic acid sequences, vectors and host cells containing them, amino acid sequences encoded by said sequences, and antibodies reactive with said amino acid sequences, as well as pharmaceutical compositions comprising any of the above. The present invention further concerns methods for screening for candidate activator or deactivators utilizing said amino acid sequences.

BACKGROUND OF THE INVENTION

Alternative splicing (AS) is an important regulatory mechanism in higher eukaryotes (P.A. Sharp, *Cell* 77, 805-8152 (1994). It is thought to be one of the important mechanisms for differential expression related to tissue or development stage specificity. It is known to play a major role in numerous biological systems, including human antibody responses, sex determination in *Drosophila*, and (S. Stamm, M.Q. Zhang, T.G. Marr and D.M. Helfman, *Nucleic Acids Research* **22**, 1515-1526 (1994); B. Chabot, *Trends Genet.* **12**, 472-478 (1996); R.E. Breitbart, A. Andreadis, B. Nadal-Ginard, *Annual Rev. Biochem.*, **56**, 467-495 (1987); C.W. Smith, J.G. Patton, B. Nadal-Ginard, *Annu. Rev. Genet.*, **27**, 527-577 (1989).

Until recently it was commonly believed that alternative splicing existed in only a small fraction of genes (about 5%). A recent observation based on literature survey of known genes revises this estimate to as high as stating that at least 30% of human genes are alternatively spliced (M.S. Gelfand, I. Dubchak, I. Draluk and M. Zorn, *Nucleic Acids Research* **27**, 301-302 (1999). The importance of the actual frequency of this phenomenon lies not only in the direct

impact on the number of proteins created (100,000 human genes, for example, would be translated to a much higher number of proteins), but also in the diversity of functionality derived from the process.

Several mechanisms at different stages may be held responsible for the complexity of higher eukaryote which include: alternative splicing at the transcription level, RNA editing at the post-transcriptional level, and post-translational modifications are the ones characterized to date.

Kinases are enzymes that catalyze the phosphorylation of target proteins. This phosphorylation event causes activation, or at times inactivation, of the target protein. Kinases are divided into two major groups, based on the amino acid residue that they phosphorylate: tyrosine kinases and the more abundant group, serine/threonine kinases play a key role in the signal transduction mechanisms that control diverse biological processes, including cellular proliferation, differentiation, adhesion, mobility, survival and apoptosis, the immune response, neutrotransmission and cellular metabolism.

Alterations in the activity of various kinases have been extensively studied and linked to the pathogenesis of most major diseases, including cancer, central nervous system disorders, immune diseases/inflammations, asthma, autoimmune disease, arthritis, graft vs. host disease and transplantation complications, cardiovascular disease, liver disease, hormonal and metabolic disorders, osteoporosis, AIDS and other infectious disease.

A dominant negative is an inactive form of a protein that reduces or eliminates the activity of its active form. It may act by binding to the active protein and rendering it inactive, or where it is an enzyme by binding the target protein without enzymatically activating the protein, thus blocking and preventing the active enzymes from binding and activating the target protein. Dominant negative kinases might be alternatively spliced gene products, which lost an important site or domain, and thus became enzymatically inactive and therefor act as inhibitors of the active kinases, for example, by binding to the target protein without phosphorylating it and blocking the binding to other active

kinases. By inhibiting the activity of the active kinases, the dominant negative kinases may interfere with a disease related process, such as cell proliferation in a tumor .Kinase variants can act as dominant-negative inhibitors through a variety of mechanisms depending on their lost or defected site or domain. For example, truncated Growth Hormone Receptor that lacks most of its intracellular domain has been shown to heterodimerize with the full-length receptor, thus causing inhibition of signaling by Growth Hormone (Ross, R.J.M., *Growth hormone & IGF Research*, **9**:42-46, 1999).

Direct specific inhibition of Protein Kinase C (PKC), for example, has been shown to initiate apoptosis in a variety of malignant cell types. Recently, two new alternatively spliced forms of PKC delta has been reported. The first – PKC delta II (mouse) – has 73 bp (26 amino acid) insertion at the caspase recognition region turning it to a caspase-resistant form.

The second – PKC delta III (rat) – has 83 bp insertion in the same region causing frame shift and forming truncated protein without the catalytic domain. That truncated form has been considered to act in a dominant-negative manner against the intact sub-type (Ueyama, T., *et al., Biochem. Biophys. Research Communications*, **269**:557-563, 2000).

Many kinase inhibitors have clinical implications. They serve as drug targets or show potential for the treatment of many diseases, such as potential in the treatment of certain cancers and treatment of type II diabetes. Tyrosine kinase inhibitors have shown various effects in treatment of neurodegenerative disease including Alzheimer's and Parkinson's disease, prevention of restenosis following angioplasty, treatment of atherosclerosis, inflammation, thrombosis, autoimmune disease, allergy, asthma, transplant rejection, psoriasis, fibrosis, dwarfism and other growth disorders, as well as inhibiting the proliferation and function of natural killer cells (immunosuppressant effect).

Protein Kinase C (PKC) inhibitors are used in cardiovascular disease, diabetes, CNS disorders, arthritis, septic shock and inflammation bowel disease,

and PKC inhibitors have shown also potential in the treatment of certain cancers and have been shown to initiate apoptosis in a variety of malignant cell types.

p38 Kinase inhibitors may be effective in attenuating COX-2-mediated prostaglandins, inflammation, treatment of rheumatoid arthritis and inflammatory bowel diseases.

GLOSSARY

In the following description and claims use will be made, at times, with a variety of terms, and the meaning of such terms as they should be construed in accordance with the invention is as follows:

"Dominant negative kinase variant (DNKV) nucleic acid sequence" – the sequence shown in any one of the sequences denoted SEQ ID NO: 1 to SEQ ID NO: 91 sequences having at least *90% identity* (see below) to said sequence and *fragments* (see below) of the above sequences of at least 20 b.p. long. These sequences are sequences coding for novel, naturally occurring, dominant negative kinase variants (DNKV) which may be obtained by alternative splicing of native and known genes. It should be emphasized that the novel variants of the present invention are naturally occurring sequences resulting from alternative splicing of genes and not merely artificially truncated, mutated or fragmented forms of known sequences. Thus the alternative dominant negative kinase variants of the invention have physiological significance as regards where, in what tissues, when, at which developmental stage and under which conditions (such as diseases, etc.) their expression is modulated, i.e., ceased, increased, up-regulated or down-regulated.

"DNKV product – also referred at times as the "DNKV protein" or ***"DNKV polypeptide"*** – is an amino acid sequence encoded by the DNKV nucleic acid sequence which is a naturally occurring mRNA sequence obtained as a result of alternative splicing. The amino acid sequence may be a peptide, a protein, as well as peptides or proteins having *chemically modified* amino acids (see below) such as

a glycopeptide or glycoprotein. The term also includes *homologues* (see below) of said sequences in which one or more amino acids has been added, deleted, *substituted* (see below) or *chemically modified* (see below) as well as *fragments* (see below) of this sequence having at least 10 amino acids. More specifically, it concerns the amino acid sequences present in SEQ ID NO: 92 to SEQ ID NO: 182.

"Nucleic acid sequence" – a sequence composed of DNA nucleotides, RNA nucleotides or a combination of both types and may include natural nucleotides, chemically modified nucleotides and synthetic nucleotides.

"Amino acid sequence" – a sequence composed of any one of the 20 naturally appearing amino acids, amino acids which have been *chemically modified* (see below), or composed of synthetic amino acids.

"Fragment of DNKV nucleic acid sequence" – novel short stretch of nucleic acid sequences of at least 20 b.p., which does not appear as a continuous stretch in the *original nucleic acid sequence* (see below). The fragment may be a sequence which was previously undescribed in the context of the published RNA and which affects the amino acid sequence encoded by the known gene. For example, where the variant nucleic includes a sequence which was not included in the original sequence (a sequence which was an intron in the original sequence) the fragment includes that additional sequence. The fragment may also be a region which is not an intron, which was not present in the original sequence. Another example is when the variant lacks a non-terminal region which was present in the original sequence. The two stretches of nucleotides spanning this region (upstream and downstream of this region) are brought together by splicing in the variant, but are spaced from each by that region in the original sequence and are thus not continuous. A continuous stretch of nucleic acids comprising said two stretches of nucleotides, is not present in the original

sequence and they are spaced at present in the variant and thus fall under the definition of fragment.

"Fragments of DNKV products" - novel amino acid sequences coded by the *"fragment of DNKV nucleic acid sequence"* defined above.

"Homologues of variants" – amino acid sequences of variants in which one or more amino acids has been added, deleted or replaced. The addition, deletion or replacement should be in regions or adjacent to regions where the variant differs from the *original sequence* (see below).

"Conservative substitution" - refers to the substitution of an amino acid in one class by an amino acid of the same class, where a class is defined by common physicochemical amino acid side chain properties and high substitution frequencies in homologous proteins found in nature, as determined, for example, by a standard Dayhoff frequency exchange matrix or BLOSUM matrix. [Six general classes of amino acid side chains have been categorized and include: Class I (Cys); Class II (Ser, Thr, Pro, Ala, Gly); Class III (Asn, Asp, Gln, Glu); Class IV (His, Arg, Lys); Class V (Ile, Leu, Val, Met); and Class VI (Phe, Tyr, Trp). For example, substitution of an Asp for another class III residue such as Asn, Gln, or Glu, is a conservative substitution.

"Non-conservative substitution" - refers to the substitution of an amino acid in one class with an amino acid from another class; for example, substitution of an Ala, a class II residue, with a class III residue such as Asp, Asn, Glu, or Gln.

"Chemically modified" - when referring to the product of the invention, means a product (protein) where at least one of its amino acid resides is modified either by natural processes, such as processing or other post-translational modifications, or by chemical modification techniques which are well known in the art. Among

the numerous known modifications typical, but not exclusive examples include: acetylation, acylation, amidation, ADP-ribosylation, glycosylation, GPI anchor formation, covalent attachment of a lipid or lipid derivative, methylation, myristlyation, pegylation, prenylation, phosphorylation, ubiqutination, or any similar process.

"Biologically active" - refers to the DNKV product having some sort of biological activity, for example, some physiologically measurable effect on target cells, molecules or tissues.

"Immunologically active" defines the capability of a natural, recombinant or synthetic DNKV product, or any fragment thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies. Thus, for example, an immunologically active fragment of DNKV product denotes a fragment which retains some or all of the immunological properties of the DNKV product, e.g can bind specific anti-DNKV product antibodies or which can elicit an immune response which will generate such antibodies or cause proliferation of specific immune cells which produce variant.

"Optimal alignment" - is defined as an alignment giving the highest percent identity score. Such alignment can be performed using a variety of commercially available sequence analysis programs, such as the local alignment program LALIGN using a ktup of 1, default parameters and the default PAM. A preferred alignment is the one performed using the CLUSTAL-W program from MacVector (TM), operated with an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM similarity matrix. If a gap needs to be inserted into a first sequence to optimally align it with a second sequence, the percent identity is calculated using only the residues that are paired with a corresponding amino acid residue (i.e., the calculation does not consider residues in the second sequences that are in the *"gap"* of the first sequence). In case of alignments of

known gene sequences with that of the new variant, the optimal alignment invariably included aligning the identical parts of both sequences together, then keeping apart and unaligned the sections of the sequences that differ one from the other.

"Having at least 90% identity" - with respect to two amino acid or nucleic acid sequence sequences, refers to the percentage of residues that are identical in the two sequences when the sequences are optimally aligned. Thus, 90% amino acid sequence identity means that 90% of the amino acids in two or more optimally aligned polypeptide sequences are identical, however this definition explicitly excludes sequences which are 100% identical with the original sequence from which the variant of the invention was varied.

"Isolated nucleic acid molecule having an DNKV nucleic acid sequence" - is a nucleic acid molecule that includes the coding DNKV nucleic acid sequence. Said isolated nucleic acid molecule may include the DNKV nucleic acid sequence as an independent insert; may include the DNKV nucleic acid sequence fused to an additional coding sequences, encoding together a fusion protein in which the DNKV coding sequence is the dominant coding sequence (for example, the additional coding sequence may code for a signal peptide); the DNKV nucleic acid sequence may be in combination with non-coding sequences, e.g., introns or control elements, such as promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host; or may be a vector in which the DNKV protein coding sequence is a heterologous.

"Expression vector" - refers to vectors that have the ability to incorporate and express heterologous DNA fragments in a foreign cell. Many prokaryotic and eukaryotic expression vectors are known and/or commercially available.

Selection of appropriate expression vectors is within the knowledge of those having skill in the art.

"Deletion" - is a change in either nucleotide or amino acid sequence in which one or more nucleotides or amino acid residues, respectively, are absent.

"Insertion" or "addition" - is that change in a nucleotide or amino acid sequence which has resulted in the addition of one or more nucleotides or amino acid residues, respectively, as compared to the naturally occurring sequence.

"Substitution" - replacement of one or more nucleotides or amino acids by different nucleotides or amino acids, respectively. As regards amino acid sequences the substitution may be conservative or non- conservative.

"Antibody" – refers to IgG, IgM, IgD, IgA, and IgG antibody. The definition includes polyclonal antibodies or monoclonal antibodies. This term refers to whole antibodies or fragments of the antibodies comprising the antigen-binding domain of the anti-variant product antibodies, e.g. antibodies without the Fc portion, single chain antibodies, fragments consisting of essentially only the variable, antigen-binding domain of the antibody, etc.

"Treating a disease" - refers to administering a therapeutic substance effective to ameliorate symptoms associated with a disease, to lessen the severity or cure the disease, or to prevent the disease from occurring. Preferably the treatment of the disease by administration of the DNKV of the invention (either the protein or the nucleic acid sequence coding for the protein) is by the inhibition of the activation of the *"original sequence"* of the *"counterpart kinase"* (see below).

"***Detection***" – refers to a method of detection of a disease, disorder, pathological or normal condition. This term may refer to detection of a predisposition to a

disease as well as for establishing the prognosis of the patient by determining the severity of the disease.

"Probe" – the DNKV nucleic acid sequence, or a sequence complementary therewith, when used to detect presence of other similar sequences in a sample. The detection is carried out by identification of hybridization complexes between the probe and the assayed sequence. The probe may be attached to a solid support or to a detectable label.

"Original sequence" **or** ***"original sequence of the counterpart kinase"*** – the amino acid or nucleic acid sequence of the kinase, from which the DNKV of the invention have been varied as a result of alternative splicing. The DNKV protein is believed to inhibit the activity of the counterpart kinase by a dominant negative mechanism as defined above.

"Data carrier" – a medium for holding informational data which is in a computer readable form. It may be a magnetic or non-magnetic data carrier.

SUMMARY OF THE INVENTION

The present invention is based on the finding of several novel, naturally occurring variants of protein kinases which lack a domain or region required for phosphorylation, and thus may be dominant negative kinases. The dominant negative kinase variants (DNKVs) of the invention are naturally occurring sequences which may be obtained by alternative splicing of known original sequences of the kinase genes. The novel DNKVs of the invention are not merely artificially truncated forms, fragments or mutations of known genes, but rather novel sequences which naturally occur within the body of individuals.

Each novel DNKV is a result of alternative splicing of an original counterpart kinase sequence. One original sequence may have one or more DNKV sequences derived therefrom by alternative splicing.

Thus, the DNKV protein of the invention may be used to inhibit the phosphorylation activity of its counterpart kinase, and thus may be used to treat diseases, wherein a beneficial therapeutical effect may be achieved by inhibiting the enzymatic activity of the counterpart kinase. The DNKV may be used as therapeutic agents which cause their beneficial effect by inhibition of kinase enzymatic activity.

The term *"alternative splicing"* in the context of the present invention and claims refers to: intron inclusion, exon exclusion, addition or deletion of terminal sequences in the DNKV as compared to the original kinase sequences.

The novel DNKV products of the invention typically have an opposite physiological activity from the activity featured by the original counterpart kinase from which they are originated (by alternative splicing), i.e. while the kinase phosphorylates proteins, the DNKVs do not have this enzymatic activity, but are able to inhibit the enzymatic activity of their counterpart kinases. Thus the DNKVs may serve as inhibitors of their counterpart kinases.

Typically the DNKV of the invention retains the binding properties of the original counterpart kinase while lacking its enzymatic activity or having a much lower level of such an activity and thus in fact neutralizes the activity of the kinase. The DNKV of the invention may further differ from the original sequence in various properties not directly connected to its biological activity such as in its stability, its clearance rate, tissue and cellular localization, its temporal pattern of expression, mechanisms for its up or down regulations, responses to agonists or antagonists, etc.

The main utility of the DNKVs of the invention is for neutralization the kinase activity of their kinase counterpart as will be explained hereinbelow.

The novel DNKVs may serve for detection purposes, i.e. their presence or level may be indicative of a disease, disorder, pathological or normal condition. Alternatively the ratio between the level variants and the level original peptide from which they were varied, or the ratio to other DNKVs (derived from the same

original sequence) may be indicative to a disease, disorder, pathological or normal condition.

For example, for detectional purposes, it is possible to establish differential expression of various DNKVs in various tissues. A certain DNKV may be expressed mainly in one tissue, while the original kinase sequence from which it has been varied, or another variant derived from the same sequence, may be expressed mainly in another tissue. Understanding of the distribution of the variants in various tissues may be helpful in basic research, for understanding the physiological function of the genes as well as may help in targeting pharmaceuticals or developing pharmaceuticals.

The study of the DNKVs may also be helpful to distinguish various stages in the life cycles of cells which may also be helpful for development of pharmaceuticals for various pathological conditions in which cell cycles is non-normal, for example cancer.

Thus the detection may by determination of the presence or the level of expression of the DNKV within a specific cell population, comprising determining said presence or level and comparing it between various cell types in a tissue, between different tissues and between individuals.

Thus the present invention provides by its first aspect, a novel isolated nucleic acid molecule comprising or consisting of any one of the coding sequence SEQ ID NO: 1 to SEQ ID NO: 91, fragments of said coding sequence having at least 20 nucleic acids (provided that said fragments are continuous stretches of nucleotides not present in the original sequence from which the variant was varied), or a molecule comprising a sequence having at least 90%, identity to SEQ ID NO: 1 to SEQ ID NO: 91, provided that the molecule is not completely identical to the original kinase sequence from which the variant was varied.

The present invention further provides a protein or polypeptide comprising or consisting of an amino acid sequence encoded by any of the above nucleic acid sequences, termed herein *"DNKV product"*, fragments of the above amino acid sequence having a length of at least 10 amino acids coded by the above fragments

of the nucleic acid sequences, as well as homologues of the above amino acid sequences in which one or more of the amino acid residues has been substituted (by conservative or non-conservative substitution) added, deleted, or chemically modified. More specifically, the amino acid sequences are those denoted as SEQ ID NO: 92 to SEQ ID NO: 182.

The deletions, insertions and modifications should be in regions, or adjacent to regions, wherein the DNKV differs from the original sequence.

For example, where the DNKV is different from the original sequence by addition of a short stretch of 10 amino acids, in the terminal or non-terminal portion of the peptide, the invention also concerns homologues of that DNKV where the additional short stretch is altered for example, it includes only 8 additional amino acids, includes 13 additional amino acids, or it includes 10 additional amino acids, however some of them being conservative or non-conservative substitutes of the original additional 10 amino acids of the novel DNKVs. In all cases the changes in the homolog, as compared to the original sequence, are in the same regions where the DNKV differs from the original kinase sequence, or in regions adjacent to said region.

Another example is where the DNKV lacks a non-terminal region (for example of 20 amino acids) which is present in the original kinase sequence (due for example to exon exclusion). The homologues may lack in the same region only 17 amino acids or 23 amino acids. Again the deletion is in the same region where the DNKV lacks a sequence as compared to the original kinase sequence, or in a region adjacent thereto.

It should be appreciated that once a man versed in the art's attention is directed to the importance of a specific region, due to the fact that this region differs in the DNKV as compared to the original kinase sequence, there is no problem in derivating said specific region by addition to it, deleting from it, or substituting some amino acids in it. Thus homologues of DNKVs which are derivated from the DNKV by changes (deletion, addition, substitution) only in said region as well as in regions adjacent to it are also a part of the present invention.

Generally, if the DNKV is distinguished from the original kinase sequence by some sort of physiological activity, then the homolog is distinguished from the original kinase sequence in essentially the same manner.

The present invention further provides nucleic acid molecule comprising or consisting of a sequence which encodes the above amino acid sequences, (including the fragments and homologues of the amino acid sequences). Due to the degenerative nature of the genetic code, a plurality of alternative nucleic acid sequences, beyond those depicted in any one of SEQ ID NO: 1 to SEQ ID NO: 91, can code for the amino acid sequence of the invention. Those alternative nucleic acid sequences which code for the same amino acid sequences coded by the sequence SEQ ID NO:1 to SEQ ID NO:91 are also an aspect of the of the present invention.

The present invention further provides expression vectors and cloning vectors comprising any of the above nucleic acid sequences, as well as host cells transfected by said vectors.

The present invention still further provides pharmaceutical compositions comprising, as an active ingredient, said nucleic acid molecules, said expression vectors, or said protein or polypeptide. The pharmaceutical composition mainly acts through inactivation of the phosphorylation activity of the counterpart kinase, and may be used to treat diseases and conditions wherein a beneficial effect can be obtained by neutralizing the activity of any one of the counterpart kinases specified under *"mRNA/Synonyms/Accession"* in Table 1 below. More specifically, the diseases are those mentioned under "*diseases*" in Table 1 below.

Alternatively, these pharmaceutical compositions are suitable for the treatment of diseases and pathological conditions, which can be ameliorated, cured or prevented by raising the level of any one of the DNKV products of the invention.

By a second aspect, the present invention provides a nucleic acid molecule comprising or consisting of a non-coding sequence which is complementary to that of any one of SEQ ID NO: 1 to SEQ ID NO: 91, or complementary to a sequence

having at least 90% identity to said sequence (with the proviso added above) or a fragment of said two sequences (according to the above definition of fragment). The complementary sequence may be a DNA sequence which hybridizes with any one of SEQ of ID NO: 1 to SEQ ID NO: 91 or hybridizes to a portion of that sequence having a length sufficient to inhibit the transcription of the complementary sequence. The complementary sequence may be a DNA sequence which can be transcribed into an mRNA being an antisense to the mRNA transcribed from any one of SEQ ID NO: 1 to SEQ ID NO: 91 or into an mRNA which is an antisense to a fragment of the mRNA transcribed from any one of SEQ ID NO: 1 to SEQ ID NO: 91 which has a length sufficient to hybridize with the mRNA transcribed from SEQ ID NO:1 to SEQ ID NO:91, so as to inhibit its translation. The complementary sequence may also be the mRNA or the fragment of the mRNA itself.

The nucleic acids of the second aspect of the invention may be used for therapeutic or diagnostic applications for example as probes used for the detection of the DNKVs of the invention.

The presence of the DNKV transcript or the level of the DNKV transcript (identified either by any one of SEQ ID NO: 1 to SEQ ID NO: 91 or by a sequence complementary thereto) may be indicative of a multitude of diseases, disorders and various pathological as well as normal conditions. In addition, the ratio of the level of the transcripts of the DNKVs of the invention may also be compared to that of the transcripts of the original kinase sequences from which they were varied, or to the level of transcript of other DNKVs, and said ratio may be indicative to a multitude of diseases, disorders and various pathological and normal conditions.

The present invention also provides expression vectors comprising any one of the above defined complementary nucleic acid sequences and host cells transfected with said nucleic acid sequences or vectors, being complementary to those specified in the first aspect of the invention.

The invention also provides anti-DNKV product antibodies, namely antibodies directed against the DNKV product which specifically bind to said

DNKV product. Said antibodies are useful both for diagnostic and therapeutic purposes. For example said antibodies may be as an active ingredient in a pharmaceutical composition as will be explained below.

By another alternative, the invention concerns antibodies termed *"distinguishing antibodies"* which are directed solely to the amino acid sequences which distinguishes the DNKV from the original amino acid sequence from which it has been varied by alternative splicing. For example, where the contains 15 additional amino acids as compared to the original kinase sequence (due to intron inclusion) the antibodies may be directed against these additional amino acids (present in the DNKV and not present in the original kinase sequence). Another example is where the DNKV lacks 20 amino acids as compared to the original kinase sequence from which it is varied (for example due to exon exclusion). The distinguishing antibodies in that case may be directed only against these 20 amino acids which are present in the original kinase sequence and absent from the DNKV sequence.

The antibodies and the distinguishing antibodies may be used for detection purposes, i.e. to detect individuals, tissue, conditions (both pathological or physiological) wherein the DNKV sequence or original kinase sequence are evident or abundant. The antibodies may also be used to distinguish conditions where the level, or ratio of the DNKV to original kinase sequence is altered.

The antibodies and the distinguishing antibodies may also be used for therapeutical purposes, i.e., to neutralize only the DNKV product or only the product of the original kinase sequence, as the case may be, without neutralizing the other.

The present invention also provides pharmaceutical compositions comprising, as an active ingredient, the nucleic acid molecules which comprise or consist of said complementary sequences, or of a vector comprising said complementary sequences. The pharmaceutical composition thus provides pharmaceutical compositions comprising, as an active ingredient, said anti-DNKV product antibodies.

The pharmaceutical compositions comprising said anti-DNKV product antibodies or the nucleic acid molecule comprising said complementary sequence, are suitable for the treatment of diseases and pathological conditions where a therapeutically beneficial effect may be achieved by neutralizing the DNKV (either at the transcript or product level) or decreasing the amount of the variant product or blocking its binding to its target, for example, by the neutralizing effect of the antibodies, or by the decrease of the effect of the antisense mRNA in decreasing expression level of the DNKV product.

The DNKV products of the invention may also be used for screening of pharmaceuticals which interact only with the DNKV and not with the original kinase sequence, or *vice versa*, thereby choosing or tailoring pharmaceuticals having better specificity either to tissues, specific conditions or better specificity to proteins expressed by a specific individual.

According to the third aspect of the invention the present invention provides methods for detecting the level of the transcript (mRNA) of said DNKV product in a body fluid sample, or in a specific tissue sample, for example by use of probes comprising or consisting of said coding sequences which can hybridize and form hybridization complexes, or by use of primers for amplification; as well as methods for detecting levels of expression of said product in tissue, e.g. by the use of antibodies capable of specifically reacting with the DNKV products of the invention. Detection of the level of the expression of the DNKV of the invention, in particular as compared to that of the original kinase sequence from which it was varied, or compared to other DNKV sequences all varied from the same original kinase sequence may be indicative of a plurality of physiological or pathological conditions.

The method, according to this latter aspect, for detection of a nucleic acid sequence which encodes the DNKV product in a biological sample, comprises the steps of:

(a) providing a probe comprising at least one of the nucleic acid sequences defined above;

(b) contacting the biological sample with said probe under conditions allowing hybridization of nucleic acid sequences thereby enabling formation of hybridization complexes;

(c) detecting hybridization complexes, wherein the presence of the complex indicates the presence of nucleic acid sequence encoding the DNKV product in the biological sample.

By another option the present invention provides a method for detection of a nucleic acid sequence in a biological sample the method comprises:

(i) contacting the sample with probes for amplification of any one of SEQ ID NO: 1 to SEQ ID NO: 91;

(ii) proving reagents for amplification;

(iii) detecting the presence of amplified products,

said products indicating the presence of DNKV nucleic acid in the sample.

The method as described above is qualitative, i.e. indicates whether the transcript is present in or absent from the sample. The method can also be quantitative, by determining the level of hybridization complexes and then calibrating said levels to determining levels of transcripts of the desired DNKV in the sample, or by determining the amount of amplified products and then calibrating said amplified amounts.

Both qualitative and quantitative determination methods can be used for diagnostic, prognostic and therapy planning purposes.

By a preferred embodiment the probe is part of a nucleic acid chip used for detection purposes, i.e. the probe is a part of an array of probes each present in a known location on a solid support.

The nucleic acid sequence used in the above method may be a DNA sequence, an RNA sequence, etc; it may be a coding or a sequence or a sequence complementary thereto (for respective detection of RNA transcripts or coding-DNA sequences). By quantization of the level of hybridization complexes and calibrating the quantified results it is possible also to detect the level of the transcript in the sample. If desired, the detected level may be compared to that of

the original kinase sequence or compared to that of other splice DNKVs, for example, those obtained from the same original kinase sequence by alternative splicing.

Methods for detecting mutations in the region coding for the DNKV product are also provided, which may be methods carried-out in a binary fashion, namely merely detecting whether there is any mismatches between the normal DNKV nucleic acid sequence of the invention and the one present in the sample, or carried-out by specifically detecting the nature and location of the mutation.

The present invention also concerns a method for detecting DNKV product in a biological sample, comprising the steps of:

(a) contacting with said biological sample the antibody of the invention, thereby forming an antibody-antigen complex; and

(b) detecting said antibody-antigen complex

wherein the presence of said antibody-antigen complex correlates with the presence of DNKV product in said biological sample.

As indicated above, the method can be quantitized to determine the level or the amount of the DNKV in the sample, alone or in comparison to the level of the original amino acid sequence from which it was varied, and qualitative and quantitative results may be used for diagnostic, prognostic and therapy planning purposes.

By yet another aspect the invention also provides a method for identifying candidate compounds capable of binding to the DNKV product and modulating its activity (being either activators or deactivators). The method includes:

(i) providing a protein or polypeptide comprising an amino acid sequence substantially as coded by any one of SEQ ID NO: 92 to 182, or a fragment of such a sequence;

(ii) contacting a candidate compound with said amino acid sequence;

(iii) measuring the physiological effect of said candidate compound on the activity of the amino acid sequences and selecting those compounds which show a significant effect on said physiological activity.

The present invention also concerns compounds identified by the above methods described above, which compound may either be an activator of the variant product or a deactivator thereof.

The detection purposes mentioned above, utilizing detection of DNKV mRNA (by hybridization or amplification) or DNKV product (by antibodies) may be used to detect a plurality of diseases. The therapeutic purposes utilizing the DNKV sequence, expression vector, antisense DNKV, DNKV product or anti-DNKV antibody may also be used to treat a plurality of diseases. For each DNKV (referred to by its SEQ ID NO) the diseases to be detected or treated are those which are involved with any one of the kinase functions mentioned for each sequence in Table 1 below. More specifically examples of the diseases are mentioned in Table 1 below.

In the following Table 1 represents the new SEQ ID NO: of the current application (mentioned as NV#), *"Nv 135619"* is the number of the same new variant as depicted in IL Application No. 135619, and *"Nv 09/724,676"* is the number of the same new variant as mentioned in US Application No. 09/724,676. Also included in Table 1 below are mRNA definitions, Synonyms, Accession No. of the mRNA of the protein, diseases and literature

Table 1

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
1	29892	21235	protein kinase/ endoribonulcease (IRE1)		AF059198	autophosphorylation activity and an endoribonuclease activity		Tirasophon et al. Genes Dev. 12 (12), 1812-1824 (1998)
2	683 30106		serine/threonine kinase RICK (RICK)	RIP2	AF027706	Regulate apoptosis induced by the CD95/Fas receptor pathway. Serine/threonine kinase.		Inohara et al. J. Biol. Chem. 273 (20), 12296-12300 (1998)
3	28225 47929	20035	mRNA for beta-adrenergic receptor kinase	BETA-ARK-1; G-PROTEIN COUPLED RECEPTOR KINASE 2; ADRBK1; GRK2; BARK1; BARK.	X61157	Specifically phosphorylates the agonist-occupied form of the beta-adrenergic. Belongs to the ser/thr family of protein kinases – family 1 of G-protein coupled receptor.	Congestive heart failure (CHF) – via the beta-adrenergic signaling cascade; Ischemia and reperfusion injury	
4	28268	20069	blk=protein tyrosine kinase [B lymphocytes, mRNA, 2608 nt]	B LYMPHOCYTE KINASE; P55-BLK	S76617	Blk may function in a signal transduction pathway that is restricted to b lymphoid cells.	Thymic lymphoma (blk controls the proliferation of B-cell)	
5	30582	21822	CDK2	P33 PROTEIN KINASE	X61622	Serine/threonine protein kinase essential for progression of the mammalian cell cycle from G1 to S phase. Interacts with cyclins a, d, or e. Activity of cdk2 is maximal during S phase and G2.	Coronary artherosclerosis, Glomerulonephritis – a CDK antagonist decreased cell proliferation and matrix production, improving renal function; Lung carcinoma	Shiffman et al. J Biol Chem. 1996 May 24;271(21):12199-204.
6	27993	19827	PHKG1	PHKG	X80590	Phosphorylase b kinase catalyzes the phosphorylation of serine in certain substrates, including troponin i. Belongs to the ser/thr family of protein kinases.	Myopathy, Psoriasis – regulation of calcium-dependent phosphorilation in the epidermis. Disturbance of PK activity may play an important role in the clinical manifestations of the disease !!	

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
7	28503	20276	(clone PK2J) CDC2-related protein kinase (PISSLRE)	CDK10	L33264	Belongs to the ser/thr family of protein kinases. Cdc2/cdkx subfamily.		
8	28504	20277	(clone PK2J) CDC2-related protein kinase (PISSLRE)	CDK10	L33264	Belongs to the ser/thr family of protein kinases. Cdc2/cdkx subfamily		
9	28508	20281	(clone PK2J) CDC2-related protein kinase (PISSLRE)	CDK10	L33264	Belongs to the ser/thr family of protein kinases. Cdc2/cdkx subfamily		
10	28313	20114	mRNA for phosphatidylinositol 3-kinase	PI3-KINASE P110; PTDINS-3-KINASE; PI3K.	Z46973	Enzymes involved in receptor signal transduction, serine/threonine kinase. Phosphorylates ptdins, ptdins4p and ptdins(4,5)p2		Volinia et al. EMBO J. 1995 Jul 17;14(14):3339-48.
11	28315	20115	mRNA for phosphatidylinositol 3-kinase		Z46973			
12	30963	22071	mRNA for cAMP-dependent protein kinase catalytic subunit type	PKA C-ALPHA	X07767 M36872	Belongs to the ser/thr family of protein kinases. Camp subfamily.	Inflammation and pain (after injury); Leukaemia; Osteosarcoma; Colon carcinoma; Wilms' tumor – PKA modulates the activity of this tumor suppressor gene. Lewis lung carcinoma – kinase inhibitors affect tumor progression. OCD – PKA has a role in the pathophysiology	

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
13	28413	20208	mRNA for protein kinase C-Epsilon	NPKC-EPSILON; PRKCE; PKCE.	X65293 S46030	This is calcium-independent, phospholipid-dependent, serine- and threonine-specific enzyme. pkc is activated by diacylglycerol which in turn phosphorylates a range of cellular proteins. pkc also serves as the receptor for phorbol esters, a class of tumor promoters.	Cardiac hypertrophy and failure; Chronic diabetes – via phosphorilation of important regulatory proteins; Skin tumor; Neuroblastoma – inhibitors inhibited uridine-induced cell differentiation !!!	Basta et al. Biochim. Biophys. Acta 1132:154-160(1992).
14	28429 48375	20224	mRNA for receptor protein tyrosine kinase	TYROSINE-PROTEIN KINASE TYRO 10; NEUROTROPHIC TYROSINE KINASE, RECEPTOR-RELATED 3	X74764	Belongs to the insulin receptor family of tyrosine-protein kinases.		Karn et al. Oncogene 8 (12), 3433-3440 (1993)
15	29504 56875	20901	mRNA for leucine zipper bearing kinase		AB001872	Association of LZK in the c-Jun amino-terminal kinase/stress-activated protein kinase pathway		Sakuma et al. J. Biol. Chem. 272 (45), 28622-28629 (1997)
16	28442		nek3 mRNA for protein kinase	NIMA-RELATED PROTEIN KINASE 3; HSPK 36	Z29067	Kinase that may play a role in mitotic regulation.	Might be candidate for usher syndrome, because of its chromosomal location.	Schultz et al. Cell Growth Differ. 4:821-830(1993).

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
17	30256	21510	JAK family protein tyrosine kinase (JAK3)	JANUS KINASE 3; JAK-3; LEUKOCYTE JANUS KINASE; L-JAK.	U09607	Tyrosine kinase of the non-receptor type, involved in the interleukin-2 and interleukin-4 signaling pathway. Phosphorylates stat6, irs1, irs2 and pi3k.	Glomerular injury (nephropathy); via signal transduction Amyotrophic lateral sclerosis (ALS) – inhibitors increased survival. Atopic dermatitis (AD) T-cell tumors (suppressed proliferation when the kinase is down-regulated) Leukaemia	Kawamura et al. Proc. Natl. Acad. Sci. U.S.A. 91:6374-6378(1994).
18	31681 48558	22609	activin type I receptor	ACTR-I; SKR1; TGF-B SUPERFAMILY RECEPTOR TYPE I; TSR-I	U14722	Receptor for activin. Belongs to the ser/thr family of protein kinases. Tgfb receptor subfamily.		Xu et al.
19	30359	21604	MEK kinase 3 mRNA	MAPK/ERK KINASE KINASE 3; MEK KINASE 3; MEKK 3	U78876	Preferentially activates p42/44 (erk2/erk1) map kinases. Belongs to the ser/thr family of protein kinases. Map kinase kinase kinase subfamily.		Ellinger et al. J. Biol. Chem. 272:2668-2674(1997).
20	30361	21606	MEK kinase 3 mRNA	MAPK/ERK KINASE KINASE 3; MEK KINASE 3; MEKK 3	U78876	Preferentially activates p42/44 (erk2/erk1) map kinases. Belongs to the ser/thr family of protein kinases. Map kinase kinase kinase subfamily.		Ellinger et al. J. Biol. Chem. 272:2668-2674(1997).

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
21	28572	20335	CDC2-related kinase (PITALRE)	C-2K; CDK9.	L25676	Member of the cyclin-dependent kinase pair (cdk9/cyclin t) complex, also called positive transcription elongation factor b (p-tefb), which is proposed to facilitate the transition from abortive to production elongation by phosphorylating the ctd (carboxy-terminal domain) of the large subunit of rna polymerase ii (rnap ii). The cdk9/cyclin k complex has also a kinase activity toward ctd of rnap ii and can substitute for p-tefb in vitro. In vitro, phosphorylates retinoblastoma and myelin basic protein.		Grana et al. Proc. Natl. Acad. Sci. U.S.A. 91:3834-3838(1994).
22	16674305 9145731		(clone PSK-J3) cyclin-dependent protein kinase	CYCLIN-DEPE NDENT KINASE 4; PSK-J3	M14505	Probably involved in the control of the cell cycle.	Oncogene in soft-tissue sarcoma; malignant melanoma; glioma; lung cancer; leukaemia; breast cancer.	Hanks et al. Proc. Natl. Acad. Sci. U.S.A. 84, 388-392 (1987)
23	16675305 9245732		(clone PSK-J3) cyclin-dependent protein kinase	CYCLIN-DEPE NDENT KINASE 4; PSK-J3	M14505	Probably involved in the control of the cell cycle.	Oncogene in soft-tissue sarcoma; malignant melanoma; glioma; lung cancer; leukaemia; breast cancer.	Hanks et al. Proc. Natl. Acad. Sci. U.S.A. 84, 388-392 (1987)
24	30252	21506	G protein-coupled receptor kinase (GRK5)	GPRK5	L15388	Specifically phosphorylates the activated forms of g protein-coupled receptors.		Kunapuli et al. Proc. Natl. Acad. Sci. U.S.A. 90 (12), 5588-5592 (1993)

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
25	30254	21508	G protein-coupled receptor kinase (GRK5)	GPRK5	L15388	Specifically phosphorylates the activated forms of g protein-coupled receptors.		Kunapuli et al. Proc. Natl. Acad. Sci. U.S.A. 90 (12), 5588-5592 (1993)
26	30255	21509	G protein-coupled receptor kinase (GRK5)		L15388	Specifically phosphorylates the activated forms of g protein-coupled receptors.		Kunapuli et al. Proc. Natl. Acad. Sci. U.S.A. 90 (12), 5588-5592 (1993)
27	29592	20987	protein kinase C-delta 13	NPKC-DELTA	L07860	This is calcium-independent, phospholipid-dependent, serine- and threonine-specific enzyme. Pkc is activated by diacylglycerol which in turn phosphorylates a range of cellular proteins. pkc also serves as the receptor for phorbol esters, a class of tumor promoters.	Mammary tumor; Ischemia (inhibited by PKC antagonists)	Aris et al. Biochim. Biophys. Acta 1174, 171-181 (1993)
28	31320	22302	phosphorylase kinase (PSK-C3)	PHK-GAMMA-T	M31606	Increasing levels of PSK-C3 mrna in the testis correlate with postnatal testicular development, suggesting possible hormonal regulation of gene transcription. Belongs to the ser/thr family of protein kinases.	Responsible for approximately 25% of cases of glycogen storage disease. affected tissues: liver only, muscle only, liver and muscle, liver and kidney, heart only.	Hanks et al. Mol. Endocrinol. 3, 110-116 (1989)
29	31562	22492	protein-tyrosine kinase (JAK1)	JANUS KINASE 1	M64174 M35203	Tyrosine kinase of the non-receptor type, involved in the ifn-alpha/beta/gamma signal pathway. Kinase partner for the interleukin (il)-2 receptor. Belongs to the janus kinases subfamily.		Wilks et al. Mol. Cell. Biol. 11:2057-2065(1991).

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
30	31712	22640	src-like kinase (slk) mRNA	P59-FYN; SYN	M14676	Implicated in the control of cell growth.	Alcoholism; autoimmune. Osteoporosis; Cancer.	Semba et al. Proc. Natl. Acad. Sci. U.S.A. 83:5459-5463(1986).
31	31723	22651	src-like kinase (slk) mRNA	P59-FYN; SYN	M14676	Implicated in the control of cell growth.	Alcoholism; autoimmune. Osteoporosis; Cancer.	Semba et al. Proc. Natl. Acad. Sci. U.S.A. 83:5459-5463(1986).
32	29889	21232	protein kinase (HSTPK13)	SERINE-THREONINE PROTEIN KINASE 13; STPK13; PLK; PLK1	L19559	May be required for cell division and may have a role during g1 or s phase.	Erythroleukaemia.	Hamanaka et al. Cell Growth Differ. 5:249-257(1994).
33	29034	20669	casein kinase I epsilon	CKI-EPSILON	L37043	Casein kinases are operationally defined by their preferential utilization of acidic Proteins such as caseins as substrates. It can phosphorylate a large number of proteins. Belongs to the ser/thr family of protein kinases. Casein kinase i subfamily.		Fish et al. J. Biol. Chem. 270:14875-14883(1995).
34	29035	20670	casein kinase I epsilon	CKI-EPSILON	L37043	Casein kinases are operationally defined by their preferential utilization of acidic Proteins such as caseins as substrates. It can phosphorylate a large number of proteins. Belongs to the ser/thr family of protein kinases. Casein kinase i subfamily.		Fish et al. J. Biol. Chem. 270:14875-14883(1995).

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
35	29036	20671	casein kinase I epsilon	CKI-EPSILON	L37043	Casein kinases are operationally defined by their preferential utilization of acidic Proteins such as caseins as substrates. It can phosphorylate a large number of proteins. Belongs to the ser/thr family of protein kinases. Casein kinase i subfamily.		Fish et al. J. Biol. Chem. 270:14875-14883(1995).
36	30573	21813	cdc2-related protein kinase (CHED)	CHOLINESTERASE-RELATED CELL DIVISION CONTROLLER; CDC2-RELATED PROTEIN KINASE 5	M80629	Controller of the mitotic cell cycle. Involved in the blood cell development.	cholinergic signals in the hematopoietic pathway – antisense oligonucleotide inhibited megakaryocyte development in bone marrow cultures.	Lapidot-Lifson et al. Proc. Natl. Acad. Sci. U.S.A. 89 (2), 579-583 (1992)
37	2682 28583	20345	CDC42-binding protein kinase beta (CDC42BPB)		AF128625	Phosphorylate nonmuscle myosin light chain, a prerequisite for the activation of actin-myosin contractility.		Moncrieff et al. Submitted (09-FEB-1999) Division of Molecular Genetics, IBLS, Glasgow University, 56 Dumbarton Road, Glasgow G11 6NU, UK
38	2683 28584	20346	CDC42-binding protein kinase beta (CDC42BPB)		AF128625	Phosphorylate nonmuscle myosin light chain, a prerequisite for the activation of actin-myosin contractility.		Moncrieff et al. Submitted (09-FEB-1999) Division of Molecular Genetics, IBLS, Glasgow University, 56 Dumbarton Road, Glasgow G11 6NU, UK

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
39	30504	21744	casein kinase I delta	CKI-DELTA	U29171	Casein kinases are operationally defined by their preferential utilization of acidic proteins such as caseins as substrates. It can phosphorylate a large number of proteins. Belongs to the ser/thr family of protein kinases. Casein kinase i subfamily.	Neurodegenerative diseases (abnormal processing of tau which involved in many diseases)	Kusuda et al. Genomics 32 (1), 140-143 (1996)
40	29500	20899	mRNA for diacylglycerol kinase delta	DIGLYCERIDE KINASE; DGK-DELTA; DAG KINASE DELTA; 80 KDA DIACYLGLYCEROL KINASE	D73409	Similar to those of the EPH family of Protein-tyrosine kinases		Sakane et al. J. Biol. Chem. 271:8394-8401(1996).
41	31626	22555	rac protein kinase alpha	RAC-PK-ALPHA; PROTEIN KINASE B; PKB	M63167	General kinase capable of phosphorilating several known proteins		Konishi et al. Biochem. Biophys. Res. Commun. 205:817-825(1994).
42	28696	20379	STE20-related protein kinase		AB013385		Dementia; parkinsonism (only potentially because of mutation locus)	
43	28704	20386	STE20-related protein kinase		AB013385		Dementia; parkinsonism (only potentially because of mutation locus)	
44	28720	20399	STE20-related protein kinase		AB013385		Dementia; parkinsonism (only potentially because of mutation locus)	

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
45	31631492 42	22560	receptor tyrosine kinase (HEK)	TYROSINE-PR OTEIN KINASE RECEPTOR ETK1	M83941	HEK is a member of the EPH/ELK family of receptor tyrosine kinases.		Wicks et al. Proc. Natl. Acad. Sci. U.S.A. 89 (5), 1611-1615 (1992)
46	31672	22601	ser-thr protein kinase PK428		U59305	Protein kinase related to the myotonic dystrophy Protein kinase family	similar to DMPK (Myotonic dystrophy)	Zhao et al. Submitted (MAY-1996) to the EMBL/GenBank/DDBJ databases.
47	30095	21393	serine threonine kinase 11 (STK11)	SERINE/THRE ONINE-PROTEI N KINASE LKB1	AF035625	May be a member of a yet unidentified signaling pathway and it may act as a tumor-suppressor.	Mutation causes the Peutz-Jeghers syndrome (PJS), a rare hereditary disease in which there is predisposition to benign and malignant tumors of many organ systems. Pjs is an autosomal-dominant disorder characterized by melanocytic macules of the lips, multiple gastrointestinal hamartomatous polyps and an increased risk for various neoplasms, including gastrointestinal cancer. Many splice variants are known.	Nezu J.; Submitted (AUG-1996) to the EMBL/GenBank/DDBJ databases.

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
48	30096	21394	serine threonine kinase 11 (STK11)	SERINE/THREONINE-PROTEIN KINASE LKB1	AF035625	May be a member of a yet unidentified signaling pathway and it may act as a tumor-suppressor.	Mutation causes the Peutz-Jeghers syndrome (PJS), a rare hereditary disease in which there is predisposition to benign and malignant tumors of many organ systems. Pjs is an autosomal-dominant disorder characterized by melanocytic macules of the lips, multiple gastrointestinal hamartomatous polyps and an increased risk for various neoplasms, including gastrointestinal cancer. Many splice variants are known.	Nezu J.; Submitted (AUG-1996) to the EMBL/GenBank/DDBJ databases.
49	28913	20573	RIP-like kinase (RIP3)		AF156884	An adaptor proteins that contain death domains. RIP3 appears to function as an intermediary in tnfalpha-induced apoptosis.		Yu, et al. Curr. Biol. 9 (10), 539-542 (1999)
50	28768	20439	IkB kinase-b (IKK-beta)		AF080158	Serine/threonine kinases which has been identified as ikappab kinase which is essential for ikappab phosphorylation and NF-kappab activation	The activity of the kinase prevents the pathogenesis of the inflammatory response.	Hu et al. Gene 222 (1), 31-40 (1998)

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
51	30947	22055	mRNA for LIMK (LIM kinase)	LIMK-1	D26309	May be a component of an intracellular signaling pathway and may be involved in brain Development. Displays serine/threonine-specific phosphorylation of myelin basic protein and Histone (mbp) in vitro.	Limk1 seems to be implicated in williams syndrome (ws), a disease characterized by impaired visuospatial constructive cognition.	Mizuno et al. Oncogene 9:1605-1612(1994).
52	30948	22056	mRNA for LIMK (LIM kinase)		D26309			
53	30896	22014	mRNA for novel protein kinase PKN	PROTEIN-KIN ASE C-RELATED KINASE 1; PROTEIN KINASE C-LIKE PKN; SERINE-THRE ONINE PROTEIN KINASE N	D26181	Can phosphorylate ribosomal protein s6. Mediates gtpase rho dependent intracellular signalling (by similarity).		Palmer et al. Eur J Biochem. 1995 Jan 15;227(1-2):344-51.
54	30900	22018	mRNA for novel protein kinase PKN	PROTEIN-KIN ASE C-RELATED KINASE 1; PROTEIN KINASE C-LIKE PKN; SERINE-THRE ONINE PROTEIN KINASE N	D26181	Can phosphorylate ribosomal protein s6. Mediates gtpase rho dependent intracellular signalling (by similarity).		Palmer et al. Eur J Biochem. 1995 Jan 15;227(1-2):344-51

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
55	29052	20685	cell cycle related kinase	CDCH; CYCLIN-DEPENDENT PROTEIN KINASE H	AF035013		cholinergic signals in the hematopoietic pathway – antisense oligonucleotide inhibited megakaryocyte development in bone marrow cultures.	Jiang Y., Zhao K.; Submitted (NOV-1997) to the EMBL/GenBank/DDBJ databases.
56	29494	20894	mRNA for ZIP-kinase	ZIPK	AB007144	Serine/threonine kinase which mediates apoptosis.		Kawai et al. Mol Cell Biol. 1998 Mar;18(3):1642-51.
57	29158	20739	diacylglycerol kinase iota (DGKi)	DGKI	AF061936	Diacylglycerol (DAG) plays a central role in both the synthesis of complex lipids and in intracellular signaling; may have important cellular functions in the retina and brain.		Bowne et al.
58	2818449423		RYK=related to receptor tyrosine kinase [hepatoma]		S59184	Potential growth factor receptor protein tyrosine kinase. The expression of this receptor tyrosine kinase in Epithelial ovarian cancer suggests that it may be involved in tumor progression, which needs further investigation.	Protooncogene for chicken sarcoma (new variant – soluble)	Wang et al. Mol Med. 1996 Mar;2(2):189-203.
59	2819149430	20001	RYK=related to receptor tyrosine kinase [hepatoma]		S59184	Potential growth factor receptor protein tyrosine kinase. The expression of this receptor tyrosine kinase in Epithelial ovarian cancer suggests that it may be involved in tumor progression, which needs further investigation.	Protooncogene for chicken sarcoma (new variant – soluble)	Wang et al. Mol Med. 1996 Mar;2(2):189-203.

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
60	31060		myotonin protein kinase=thymopoietin homolog [muscle]	MYOTONIC DISTROPHY PROTEIN KINASE; MDPK; DM-KINASE; DMK; DMPK; MT-PK	S72883	May play a role in the intracellular communication.	Defects in dmpk are the cause of myotonic distrophy (dm), an autosomal dominant neurodegenerative disorder characterized by myotonia, muscle wasting in the distal extremities, cataract, hypogonadism, defective endocrine functions, male baldness, and cardiac arrhythmias. DM patients show decreased levels of kinase expression inversely related to repeat length. The minimum estimated incidence is 1 in 8000.	Fu et al. Science 255:1256-1258(1992).

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
61	31084		myotonin protein kinase=thymopoietin homolog [muscle]	MYOTONIC DISTROPHY PROTEIN KINASE; MDPK; DM-KINASE; DMK; DMPK; MT-PK	S72883	May play a role in the intracellular communication.	Defects in dmpk are the cause of myotonic distrophy (dm), an autosomal dominant neurodegenerative disorder characterized by myotonia, muscle wasting in the distal extremities, cataract, hypogonadism, defective endocrine functions, male baldness, and cardiac arrhythmias. DM patients show decreased levels of kinase expression inversely related to repeat length. The minimum estimated incidence is 1 in 8000.	Fu et al. Science 255:1256-1258(1992).

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
62	2856654895		ATP sulfurylase/APS kinase	PAPS SYNTHETHASE 1; PAPSS 1; SULFURYLASE KINASE 1; SK1	AF016496	Bifunctional enzyme with both atp sulfurylase and aps kinase activity, which mediates two steps in the sulfate activation pathway. The first step is the transfer of a sulfate group to atp to yield adenosine 5'-phosphosulfate (aps), and the second step is the transfer of a phosphate group from atp to aps yielding 3'-phosphoadenylylsulfate (paps: activated sulfate donor used by sulfotransferase). In mammals, paps is the sole source of sulfate; aps appears to be only an intermediate in the sulfate-activation pathway. Also involved in the biosynthesis of sulfated l-selectin ligands in endothelial cells.		Li et al. J. Biol. Chem. 270:29453-29459(1995).
63	28524	20294	3-phosphoinositide dependent protein kinase-1 (PDK1)		AF017995			Alessi et al. Curr. Biol. 7:261-269(1997).
64	29614		AKT3 protein kinase	RAC-PK-GAMMA; PROTEIN KINASE AKT-3; PROTEIN KINASE B, GAMMA; PKB GAMMA	AF135794	Igf-1 leads to the activation of akt3, which may play a role in regulating cell survival. Capable of phosphorylating several known proteins.		Nakatani et al. Biochem. Biophys. Res. Commun. 257:906-910(1999).

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
65	29615		AKT3 protein kinase	RAC-PK-GAMMA; PROTEIN KINASE AKT-3; PROTEIN KINASE B, GAMMA; PKB GAMMA	AF135794	Igf-1 leads to the activation of akt3, which may play a role in regulating cell survival. Capable of phosphorylating several known proteins.		Nakatani et al. Biochem. Biophys. Res. Commun. 257:906-910(1999).
66	29620	21010	AKT3 protein kinase	RAC-PK-GAMMA; PROTEIN KINASE AKT-3; PROTEIN KINASE B, GAMMA; PKB GAMMA	AF135794	Igf-1 leads to the activation of akt3, which may play a role in regulating cell survival. Capable of phosphorylating several known proteins.		Nakatani et al. Biochem. Biophys. Res. Commun. 257:906-910(1999).
67	30109	21401	serum-inducible kinase		AF059617			Ouyang et al. Oncogene 18 (44), 6029-6036 (1999)
68	30111	21403	serum-inducible kinase		AF059617			Ouyang et al. Oncogene 18 (44), 6029-6036 (1999)
69	30112	21404	serum-inducible kinase		AF059617			Ouyang et al. Oncogene 18 (44), 6029-6036 (1999)
70			myristilated and palmitylated serine-threonine kinase		AF060798			Berson A.E.; Submitted (APR-1998) to the EMBL/GenBank/DDBJ databases.

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
71	2916067864	20741	eukaryotic translation initiation factor 2 alpha kinase PEK mRNA		AF110146		Mutations may cause the Wolcott-Rallison syndrome.	Shi et al. J. Biol. Chem. 274:5723-5730(1999).
72	30374	21619	activated p21cdc42Hs kinase (ack)		L13738	signal transduction		Manser et al. Nature 363 (6427), 364-367 (1993)
73	30904	22022	lipid-activated, protein kinase PRK2	PROTEIN-KINASE C-RELATED KINASE 2	U33052	Exhibits a preference for highly basic protein substrates (by similarity).		Palmer et al. Eur. J. Biochem. 227 (1-2), 344-351 (1995)
74	30905	22023	lipid-activated, protein kinase PRK2	PROTEIN-KINASE C-RELATED KINASE 2	U33052	Exhibits a preference for highly basic protein substrates (by similarity).		Palmer et al. Eur. J. Biochem. 227 (1-2), 344-351 (1995)
75	29406	20850	mRNA for MNK1		AB000409	MAP kinase-activated protein kinase		Fukunaga et al. EMBO J. 16:1921-1933(1997).
76	29408	20852	mRNA for MNK1		AB000409	MAP kinase-activated protein kinase		Fukunaga et al. EMBO J. 16:1921-1933(1997).
77	29414	20857	mRNA for MNK1		AB000409	MAP kinase-activated protein kinase		Fukunaga et al. EMBO J. 16:1921-1933(1997).
78	28928		STE20-like kinase (JIK)		AF179867			Tassi et al. J. Biol. Chem. 274:33287-33295(1999).
79	30124	21411	tousled-like kinase 2 (TLK2)		AF162667	serine/threonine protein kinase		Sillje et al. EMBO J. 18:5691-5702(1999).
80	29341	20805	interleukin-1 receptor-associated kinase (IRAK)		L76191	Involved in il-1 pathway. This kinase associates with the il-1 receptor il1-r-1. This association is rapid and il-1 dependent.		Cao et al. Science 271:1128-1131(1996).

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
81	29344	20808	interleukin-1 receptor-associated kinase (IRAK)		L76191	Involved in il-1 pathway. This kinase associates with the il-1 receptor il1-r-1. This association is rapid and il-1 dependent.		Cao et al. Science 271:1128-1131(1996).
82	29349	20812	interleukin-1 receptor-associated kinase (IRAK)		L76191	Involved in il-1 pathway. This kinase associates with the il-1 receptor il1-r-1. This association is rapid and il-1 dependent.		Cao et al. Science 271:1128-1131(1996).
83	30850	21975	insulin-stimulated protein kinase 1 (ISPK-1)	S6K-ALPHA 3; 90 KDA RIBOSOMAL PROTEIN S6 KINASE 3; P90-RSK 3; RIBOSOMAL S6 KINASE 2; RSK-2; PP90RSK2	U08316	Phosphorylates a wide range of substrates including ribosomal protein s6. Implicated in the activation of the mitogen-activated kinase cascade.	Defects in rps6ka3 are the cause of coffin-lowry syndrome (cls). The features of this diseases are severe mental retardation with facial and digital dysmorphisms, and progressive skeletal deformations.	Bjoerbaek et al. Diabetes 44:90-97(1995).
84	30863	21988	insulin-stimulated protein kinase 1 (ISPK-1)	S6K-ALPHA 3; 90 KDA RIBOSOMAL PROTEIN S6 KINASE 3; P90-RSK 3; RIBOSOMAL S6 KINASE 2; RSK-2; PP90RSK2	U08316	Phosphorylates a wide range of substrates including ribosomal protein s6. Implicated in the activation of the mitogen-activated kinase cascade.	Defects in rps6ka3 are the cause of coffin-lowry syndrome (cls). The features of this diseases are severe mental retardation with facial and digital dysmorphisms, and progressive skeletal deformations.	Bjoerbaek et al. Diabetes 44:90-97(1995).

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
85	31044	22147	mitogen-activated kinase kinase kinase 5 (MAPKKK5)	MAPK/ERK KINASE KINASE 5; MEK KINASE 5; MEKK 5; APOPTOSIS SIGNAL-REGULATING KINASE 1; ASK-1	U67156	Phosphorylates and activates two different subgroups of map kinase kinases, mkk4/sek1 and mkk3/mapkk6 (or mkk6), which in turn activated stress-activated protein kinase (sapk, also known as jnk; c-jun amino-terminal kinase) and p38 subgroups of map kinases, respectively. Overexpression induces apoptotic cell death.	Genotoxic stress apoptosis (one step of the cascade of activating caspase protease); Apoptosis (sympathetic neurons).	Wang et al. J. Biol. Chem. 271:31607-31611(1996).
86	6891 28947	20594	TANK binding kinase TBK1 (TBK1)	NF-KB-ACTIVATING KINASE NAK	AF191838			Pomerantz et al. EMBO J. 18:6694-6704(1999).
87	30263	21515	MAP kinase	STRESS-ACTIVATED PROTEIN KINASE JNK3; C-JUN N-TERMINAL KINASE 3; MAP KINASE P49 3F12	U07620	Binds to the amino terminal activation domains of c-jun or atf2 and phosphorylates their regulatory sites (respectively ser-63 and ser-73/thr-69 and thr-71). Also phosphorylates elk1.		Mohit et al. Neuron 14:67-78(1995).
88	30270	21519	MAP kinase	STRESS-ACTIVATED PROTEIN KINASE JNK3; C-JUN N-TERMINAL KINASE 3; MAP KINASE P49 3F12	U07620	Binds to the amino terminal activation domains of c-jun or atf2 and phosphorylates their regulatory sites (respectively ser-63 and ser-73/thr-69 and thr-71). Also phosphorylates elk1.		Mohit et al. Neuron 14:67-78(1995).

Nv #	Nv – 135619	Nv – 09/724,676	mRNA	Synonyms	Accession	Function	Potential Diseases	Literature
89	28197	20007	mRNA for Ndr protein kinase		Z35102	Serine(threonine) protein kinase		Millward et al. Proc Natl Acad Sci U S A. 1995 May 23;92(11):5022-6.
90	28935	20592	Ste-20 related kinase SPAK		AF099989	May act as a mediator of stress-activated signals. Belongs to the dck/dgk family		Johnston et al. Oncogene 19:4290-4297(2000).
91	28936	20593	Ste-20 related kinase SPAK		AF099989	May act as a mediator of stress-activated signals. Belongs to the dck/dgk family		Johnston et al. Oncogene 19:4290-4297(2000).

The pharmaceutical compositions, whether comprising the nucleic acid sequences of the DNVKs of the invention themselves (alone or in an expression vector), comprising complementary sequences thereto (alone or in an expression vector), comprising the amino acid (products), or alternatively, comprising antibodies to the above, are suitable for the treatment of a plurality of diseases, each one in accordance with the activity of the functional group to which the new DNKV falls as mentioned in Table 1 above.

The detection of diseases utilizing a DNKV probe (comprising the DNKV sequence or a sequence complementary thereto) or alternatively comprising an amino acid sequence reactive with the DNKV product is also in accordance with the functional group to which the DNKVs belong.

Thus, in the above Table 1, there is a brief summary of those conditions, and diseases in which the pharmaceutical composition can treat, i.e. cure, ameliorate or prevent, as well as those conditions which can be detected by DNKV probes of the present invention, or by antibodies reactive with the DNKV product of the invention.

The present invention further concerns any one of SEQ ID NO: 1 to SEQ ID NO: 91 present on a data carrier. The invention further concerns the amino acid sequences present on a data carrier.

The present invention further concerns such a data carrier for use in an analysis of a nucleic acid sequence or amino acid sequence. For the purpose of the analysis said nucleic acid sequence is compared to a sequence of a plurality of nucleic acid sequences being substantially SEQ ID NO: 1 to SEQ ID NO: 91 of which are present on a data carrier or alternatively to the plurality of amino acid sequences present on the carrier. Thus, the data carrier of the invention may be used by others for analysis of nucleic acid sequences which they have, in order to determine whether the sequence they have is a sequence of splice variants of a known gene, obtained through alternative splicing.

This may be done by using a software data combination comprising a nucleotide search and comparison software and a data carrier comprising all of the

DNKV sequences of the invention. When the combination is loaded into the computer it can execute a search where a nucleotide sequence entered by the user is compared to the plurality of sequences comprising said data.

The software used for search and comparison between nucleic acid sequences is in combination with the data of the invention, may be any software known in the art for finding homology, at a specified level between an entered nucleic acid sequence and a plurality of nucleic acid sequences present on a data base any person wishing to determine whether a nucleic acid sequence he has is a splice variant of one of the original sequence, may do so by determining whether it appears in one of the sequences of the invention.

The present invention further concerns an inhibitor of kinase activity being any of the amino acid sequences coded by SEQ ID NO: 92 to SEQ ID NO: 182 or fragments thereof.

The present invention also concerns an inhibitor of kinase activity by an isolated nucleic acid sequence being any of the nucleic acid sequences of SEQ ID NO: 1 to SEQ ID NO: 91 and fragments thereof or complementary sequences to any of the above.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows an alignment between the amino acid sequence of SEQ ID NO. 92 and the amino acid sequence of the counterpart kinase.

Fig. 2 shows an alignment between the amino acid sequence of SEQ ID NO. 93 and the amino acid sequence of the counterpart kinase.

Fig. 3 shows an alignment between the amino acid sequence of SEQ ID NO. 94 and the amino acid sequence of the counterpart kinase.

Fig. 4 shows an alignment between the amino acid sequence of SEQ ID NO. 95 and the amino acid sequence of the counterpart kinase.

Fig. 5 shows an alignment between the amino acid sequence of SEQ ID NO. 96 and the amino acid sequence of the counterpart kinase.

Fig. 6 shows an alignment between the amino acid sequence of SEQ ID NO. 97 and the amino acid sequence of the counterpart kinase.

Fig. 7 shows an alignment between the amino acid sequence of SEQ ID NO. 98 and the amino acid sequence of the counterpart kinase.

Fig. 8 shows an alignment between the amino acid sequence of SEQ ID NO. 99 and the amino acid sequence of the counterpart kinase.

Fig. 9 shows an alignment between the amino acid sequence of SEQ ID NO. 100 and the amino acid sequence of the counterpart kinase.

Fig. 10 shows an alignment between the amino acid sequence of SEQ ID NO. 101 and the amino acid sequence of the counterpart kinase.

Fig. 11 shows an alignment between the amino acid sequence of SEQ ID NO. 102 and the amino acid sequence of the counterpart kinase.

Fig. 12 shows an alignment between the amino acid sequence of SEQ ID NO. 103 and the amino acid sequence of the counterpart kinase.

Fig. 13 shows an alignment between the amino acid sequence of SEQ ID NO. 104 and the amino acid sequence of the counterpart kinase.

Fig. 14 shows an alignment between the amino acid sequence of SEQ ID NO. 105 and the amino acid sequence of the counterpart kinase.

Fig. 15 shows an alignment between the amino acid sequence of SEQ ID NO. 106 and the amino acid sequence of the counterpart kinase.

Fig. 16 shows an alignment between the amino acid sequence of SEQ ID NO. 107 and the amino acid sequence of the counterpart kinase.

Fig. 17 shows an alignment between the amino acid sequence of SEQ ID NO. 108 and the amino acid sequence of the counterpart kinase.

Fig. 18 shows an alignment between the amino acid sequence of SEQ ID NO. 109 and the amino acid sequence of the counterpart kinase.

Fig. 19 shows an alignment between the amino acid sequence of SEQ ID NO. 110 and the amino acid sequence of the counterpart kinase.

Fig. 20 shows an alignment between the amino acid sequence of SEQ ID NO. 111 and the amino acid sequence of the counterpart kinase.

Fig. 21 shows an alignment between the amino acid sequence of SEQ ID NO. 112 and the amino acid sequence of the counterpart kinase.

Fig. 22 shows an alignment between the amino acid sequence of SEQ ID NO. 113 and the amino acid sequence of the counterpart kinase.

Fig. 23 shows an alignment between the amino acid sequence of SEQ ID NO. 114 and the amino acid sequence of the counterpart kinase.

Fig. 24 shows an alignment between the amino acid sequence of SEQ ID NO. 115 and the amino acid sequence of the counterpart kinase.

Fig. 25 shows an alignment between the amino acid sequence of SEQ ID NO. 116 and the amino acid sequence of the counterpart kinase.

Fig. 26 shows an alignment between the amino acid sequence of SEQ ID NO. 117 and the amino acid sequence of the counterpart kinase.

Fig. 27 shows an alignment between the amino acid sequence of SEQ ID NO. 118 and the amino acid sequence of the counterpart kinase.

Fig. 28 shows an alignment between the amino acid sequence of SEQ ID NO. 119 and the amino acid sequence of the counterpart kinase.

Fig. 29 shows an alignment between the amino acid sequence of SEQ ID NO. 120 and the amino acid sequence of the counterpart kinase.

Fig. 30 shows an alignment between the amino acid sequence of SEQ ID NO. 121 and the amino acid sequence of the counterpart kinase.

Fig. 31 shows an alignment between the amino acid sequence of SEQ ID NO. 122 and the amino acid sequence of the counterpart kinase.

Fig. 32 shows an alignment between the amino acid sequence of SEQ ID NO. 123 and the amino acid sequence of the counterpart kinase.

Fig. 33 shows an alignment between the amino acid sequence of SEQ ID NO. 124 and the amino acid sequence of the counterpart kinase.

Fig. 34 shows an alignment between the amino acid sequence of SEQ ID NO. 125 and the amino acid sequence of the counterpart kinase.

Fig. 35 shows an alignment between the amino acid sequence of SEQ ID NO. 126 and the amino acid sequence of the counterpart kinase.

Fig. 36 shows an alignment between the amino acid sequence of SEQ ID NO. 127 and the amino acid sequence of the counterpart kinase.

Fig. 37 shows an alignment between the amino acid sequence of SEQ ID NO. 128 and the amino acid sequence of the counterpart kinase.

Fig. 38 shows an alignment between the amino acid sequence of SEQ ID NO. 129 and the amino acid sequence of the counterpart kinase.

Fig. 39 shows an alignment between the amino acid sequence of SEQ ID NO. 130 and the amino acid sequence of the counterpart kinase.

Fig. 40 shows an alignment between the amino acid sequence of SEQ ID NO. 131 and the amino acid sequence of the counterpart kinase.

Fig. 41 shows an alignment between the amino acid sequence of SEQ ID NO. 132 and the amino acid sequence of the counterpart kinase.

Fig. 42 shows an alignment between the amino acid sequence of SEQ ID NO. 133 and the amino acid sequence of the counterpart kinase.

Fig. 43 shows an alignment between the amino acid sequence of SEQ ID NO. 134 and the amino acid sequence of the counterpart kinase.

Fig. 44 shows an alignment between the amino acid sequence of SEQ ID NO. 135 and the amino acid sequence of the counterpart kinase.

Fig. 45 shows an alignment between the amino acid sequence of SEQ ID NO. 136 and the amino acid sequence of the counterpart kinase.

Fig. 46 shows an alignment between the amino acid sequence of SEQ ID NO. 137 and the amino acid sequence of the counterpart kinase.

Fig. 47 shows an alignment between the amino acid sequence of SEQ ID NO. 138 and the amino acid sequence of the counterpart kinase.

Fig. 48 shows an alignment between the amino acid sequence of SEQ ID NO. 139 and the amino acid sequence of the counterpart kinase.

Fig. 49 shows an alignment between the amino acid sequence of SEQ ID NO. 140 and the amino acid sequence of the counterpart kinase.

Fig. 50 shows an alignment between the amino acid sequence of SEQ ID NO. 141 and the amino acid sequence of the counterpart kinase.

Fig. 51 shows an alignment between the amino acid sequence of SEQ ID NO. 142 and the amino acid sequence of the counterpart kinase.

Fig. 52 shows an alignment between the amino acid sequence of SEQ ID NO. 143 and the amino acid sequence of the counterpart kinase.

Fig. 53 shows an alignment between the amino acid sequence of SEQ ID NO. 144 and the amino acid sequence of the counterpart kinase.

Fig. 54 shows an alignment between the amino acid sequence of SEQ ID NO. 145 and the amino acid sequence of the counterpart kinase.

Fig. 55 shows an alignment between the amino acid sequence of SEQ ID NO. 146 and the amino acid sequence of the counterpart kinase.

Fig. 56 shows an alignment between the amino acid sequence of SEQ ID NO. 147 and the amino acid sequence of the counterpart kinase.

Fig. 57 shows an alignment between the amino acid sequence of SEQ ID NO. 148 and the amino acid sequence of the counterpart kinase.

Fig. 58 shows an alignment between the amino acid sequence of SEQ ID NO. 149 and the amino acid sequence of the counterpart kinase.

Fig. 59 shows an alignment between the amino acid sequence of SEQ ID NO. 150 and the amino acid sequence of the counterpart kinase.

Fig. 60 shows an alignment between the amino acid sequence of SEQ ID NO. 151 and the amino acid sequence of the counterpart kinase.

Fig. 61 shows an alignment between the amino acid sequence of SEQ ID NO. 152 and the amino acid sequence of the counterpart kinase.

Fig. 62 shows an alignment between the amino acid sequence of SEQ ID NO. 153 and the amino acid sequence of the counterpart kinase.

Fig. 63 shows an alignment between the amino acid sequence of SEQ ID NO. 154 and the amino acid sequence of the counterpart kinase.

Fig. 64 shows an alignment between the amino acid sequence of SEQ ID NO. 155 and the amino acid sequence of the counterpart kinase.

Fig. 65 shows an alignment between the amino acid sequence of SEQ ID NO. 156 and the amino acid sequence of the counterpart kinase.

Fig. 66 shows an alignment between the amino acid sequence of SEQ ID NO. 157 and the amino acid sequence of the counterpart kinase.

Fig. 67 shows an alignment between the amino acid sequence of SEQ ID NO. 158 and the amino acid sequence of the counterpart kinase.

Fig. 68 shows an alignment between the amino acid sequence of SEQ ID NO. 159 and the amino acid sequence of the counterpart kinase.

Fig. 69 shows an alignment between the amino acid sequence of SEQ ID NO. 160 and the amino acid sequence of the counterpart kinase.

Fig. 70 shows an alignment between the amino acid sequence of SEQ ID NO. 161 and the amino acid sequence of the counterpart kinase.

Fig. 71 shows an alignment between the amino acid sequence of SEQ ID NO. 162 and the amino acid sequence of the counterpart kinase.

Fig. 72 shows an alignment between the amino acid sequence of SEQ ID NO. 163 and the amino acid sequence of the counterpart kinase.

Fig. 73 shows an alignment between the amino acid sequence of SEQ ID NO. 164 and the amino acid sequence of the counterpart kinase.

Fig. 74 shows an alignment between the amino acid sequence of SEQ ID NO. 165 and the amino acid sequence of the counterpart kinase.

Fig. 75 shows an alignment between the amino acid sequence of SEQ ID NO. 166 and the amino acid sequence of the counterpart kinase.

Fig. 76 shows an alignment between the amino acid sequence of SEQ ID NO. 167 and the amino acid sequence of the counterpart kinase.

Fig. 77 shows an alignment between the amino acid sequence of SEQ ID NO. 168 and the amino acid sequence of the counterpart kinase.

Fig. 78 shows an alignment between the amino acid sequence of SEQ ID NO. 169 and the amino acid sequence of the counterpart kinase.

Fig. 79 shows an alignment between the amino acid sequence of SEQ ID NO. 170 and the amino acid sequence of the counterpart kinase.

Fig. 80 shows an alignment between the amino acid sequence of SEQ ID NO. 171 and the amino acid sequence of the counterpart kinase.

Fig. 81 shows an alignment between the amino acid sequence of SEQ ID NO. 172 and the amino acid sequence of the counterpart kinase.

Fig. 82 shows an alignment between the amino acid sequence of SEQ ID NO. 173 and the amino acid sequence of the counterpart kinase.

Fig. 83 shows an alignment between the amino acid sequence of SEQ ID NO. 174 and the amino acid sequence of the counterpart kinase.

Fig. 84 shows an alignment between the amino acid sequence of SEQ ID NO. 175 and the amino acid sequence of the counterpart kinase.

Fig. 85 shows an alignment between the amino acid sequence of SEQ ID NO. 176 and the amino acid sequence of the counterpart kinase.

Fig. 86 shows an alignment between the amino acid sequence of SEQ ID NO. 177 and the amino acid sequence of the counterpart kinase.

Fig. 87 shows an alignment between the amino acid sequence of SEQ ID NO. 178 and the amino acid sequence of the counterpart kinase.

Fig. 88 shows an alignment between the amino acid sequence of SEQ ID NO. 179 and the amino acid sequence of the counterpart kinase.

Fig. 89 shows an alignment between the amino acid sequence of SEQ ID NO. 180 and the amino acid sequence of the counterpart kinase.

Fig. 90 shows an alignment between the amino acid sequence of SEQ ID NO. 181 and the amino acid sequence of the counterpart kinase.

Fig. 91 shows an alignment between the amino acid sequence of SEQ ID NO. 182 and the amino acid sequence of the counterpart kinase.

INSA1

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1: DNKV nucleic acid sequence

The nucleic acid sequences of the invention include nucleic acid sequences which encode DNKV product and fragments and analogs thereof. The nucleic acid sequences may alternatively be sequences complementary to the above coding sequence, or to a region of said coding sequence. The length of the

complementary sequence is sufficient to avoid the expression of the coding sequence. The nucleic acid sequences may be in the form of RNA or in the form of DNA, and include messenger RNA, synthetic RNA and DNA, cDNA, and genomic DNA. The DNA may be double-stranded or single-stranded, and if single-stranded may be the coding strand or the non-coding (anti-sense, complementary) strand. The nucleic acid sequences may also both include dNTPs, rNTPs as well as non naturally occurring sequences. The sequence may also be a part of a hybrid between an amino acid sequence and a nucleic acid sequence.

In a general embodiment, the nucleic acid sequence has at least 90%, identity, preferably 95% with any one of the sequence identified as SEQ ID NO: 1 to SEQ ID NO: 91 provided that this sequence is not completely identical with that of the original sequence.

The nucleic acid sequences may include the coding sequence by itself. By another alternative the coding region may be in combination with additional coding sequences, such as those coding for fusion protein or signal peptides, in combination with non-coding sequences, such as introns and control elements, promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host, and/or in a vector or host environment in which the DNKV nucleic acid sequence is introduced as a heterologous sequence.

The nucleic acid sequences of the present invention may also have the product coding sequence fused in-frame to a marker sequence which allows for purification of the DNKV product. The marker sequence may be, for example, a hexahistidine tag to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., *et al. Cell* **37**:767 (1984)).

Also included in the scope of the invention are fragments as defined above also referred to herein as oligonucleotides, typically having at least 20 bases, preferably 20-30 bases corresponding to a region of the coding-sequence nucleic acid sequence. The fragments may be used as probes, primers, and when complementary also as antisense agents, and the like, according to known methods.

As indicated above, the nucleic acid sequence may be substantially a depicted in any one of SEQ ID NO: 1 to SEQ ID NO: 91 or fragments thereof or sequences having at least 90% identity to the above sequence as explained above. Alternatively, due to the degenerative nature of the genetic code, the sequence may be a sequence coding for any one of the amino acid sequence depicted in SEQ ID NO: 92 to SEQ ID NO: 182, or fragments or analogs of said amino acid sequence.

A. Preparation of nucleic acid sequences

The nucleic acid sequences may be obtained by screening cDNA libraries using oligonucleotide probes which can hybridize to or PCR-amplify nucleic acid sequences which encode the DNKV products disclosed above. cDNA libraries prepared from a variety of tissues are commercially available and procedures for screening and isolating cDNA clones are well-known to those of skill in the art. Such techniques are described in, for example, Sambrook *et al.* (1989) Molecular Cloning: A Laboratory Manual (2nd Edition), Cold Spring Harbor Press, Plainview, N.Y. and Ausubel FM et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y.

The nucleic acid sequences may be extended to obtain upstream and downstream sequences such as promoters, regulatory elements, and 5' and 3' untranslated regions (UTRs). Extension of the available transcript sequence may be performed by numerous methods known to those of skill in the art, such as PCR or primer extension (Sambrook *et al., supra*), or by the RACE method using, for example, the Marathon RACE kit (Clontech, Cat. # K1802-1).

Alternatively, the technique of *"restriction-site"* PCR (Gobinda *et al. PCR Methods Applic.* **2**:318-22, (1993)), which uses universal primers to retrieve flanking sequence adjacent a known locus, may be employed. First, genomic DNA is amplified in the presence of primer to a linker sequence and a primer specific to the known region. The amplified sequences are subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR can be used to amplify or extend sequences using divergent primers based on a known region (Triglia, T. *et al.*, *Nucleic Acids Res.* **16**:8186, (1988)). The primers may be designed using OLIGO(R) 4.06 Primer Analysis Software (1992; National Biosciences Inc, Plymouth, Minn.), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Capture PCR (Lagerstrom, M. *et al., PCR Methods Applic.* **1**:111-19, (1991)) is a method for PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA. Capture PCR also requires multiple restriction enzyme digestions and ligations to place an engineered double-stranded sequence into a flanking part of the DNA molecule before PCR.

Another method which may be used to retrieve flanking sequences is that of Parker, J.D., *et al., Nucleic Acids Res.*, **19**:3055-60, (1991)). Additionally, one can use PCR, nested primers and PromoterFinder™ libraries to *"walk in"* genomic DNA (PromoterFinder™; Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions. Preferred libraries for screening for full length cDNAs are ones that have been size-selected

to include larger cDNAs. Also, random primed libraries are preferred in that they will contain more sequences which contain the 5' and upstream regions of genes.

A randomly primed library may be particularly useful if an oligo d(T) library does not yield a full-length cDNA. Genomic libraries are useful for extension into the 5' nontranslated regulatory region.

The nucleic acid sequences and oligonucleotides of the invention can also be prepared by solid-phase methods, according to known synthetic methods. Typically, fragments of up to about 100 bases are individually synthesized, then joined to form continuous sequences up to several hundred bases.

B. Use of DNKV nucleic acid sequence for the production of DNKV products

In accordance with the present invention, nucleic acid sequences specified above may be used as recombinant DNA molecules that direct the expression of DNKV products.

As will be understood by those of skill in the art, it may be advantageous to produce DNKV product-encoding nucleotide sequences possessing codons other than those which appear in any one of SEQ ID NO: 1 to SEQ ID NO: 91 which are those which naturally occur in the human genome. Codons preferred by a particular prokaryotic or eukaryotic host (Murray, E. *et al. Nuc Acids Res.*, **17**:477-508, (1989)) can be selected, for example, to increase the rate of DNKV product expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence.

The nucleic acid sequences of the present invention can be engineered in order to alter a DNKV product coding sequence for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the product. For example, alterations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis,

to insert new restriction sites, to alter glycosylation patterns, to change codon preference, etc.

The present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a nucleic acid sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are also described in Sambrook, *et al., (supra)*.

The present invention also relates to host cells which are genetically engineered with vectors of the invention, and the production of the product of the invention by recombinant techniques. Host cells are genetically engineered (i.e., transduced, transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the expression of the DNKV nucleic acid sequence. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art.

The nucleic acid sequences of the present invention may be included in any one of a variety of expression vectors for expressing a product. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and related sub-cloning procedures are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate transcription control sequence (promoter) to direct mRNA synthesis. Examples of such promoters include: LTR or SV40 promoter, the *E.coli lac* or *trp* promoter, the phage lambda *PL* promoter, and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation, and a transcription terminator. The vector may also include appropriate sequences for amplifying expression. In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E.coli.*

The vector containing the appropriate DNA sequence as described above, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein. Examples of appropriate expression hosts include: bacterial cells, such as *E.coli, Streptomyces, Salmonella typhimurium*; fungal cells, such as yeast; insect cells such as *Drosophila* and *Spodoptera* Sf9; animal cells such as CHO, COS, HEK 293 or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein. The invention is not limited by the host cells employed.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for the DNKV product. For example, when large quantities of DNKV product are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily

purified may be desirable. Such vectors include, but are not limited to, multifunctional *E.coli* cloning and expression vectors such as *Bluescript*(R) (Stratagene), in which the DNKV polypeptide coding sequence may be ligated into the vector in-frame with sequences for the amino-terminal Met and the subsequent 7 residues of beta-galactosidase so that a hybrid protein is produced; *pIN* vectors (Van Heeke & Schuster *J. Biol. Chem.* **264**:5503-5509, (1989)); *pET* vectors (Novagen, Madison WI); and the like.

In the yeast *Saccharomyces cerevisiae* a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase and PGH may be used. For reviews, see Ausubel *et al. (supra)* and Grant *et al.*, (*Methods in Enzymology* **153**:516-544, (1987)).

In cases where plant expression vectors are used, the expression of a sequence encoding DNKV product may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of *CaMV* (Brisson *et al., Nature* **310**:511-514. (1984)) may be used alone or in combination with the omega leader sequence from TMV (Takamatsu *et al.*, *EMBO J.*, **6**:307-311, (1987)). Alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi *et al.*, *EMBO J.* **3**:1671-1680, (1984); Broglie *et al.*, *Science* **224**:838-843, (1984)); or heat shock promoters (Winter J and Sinibaldi R.M., *Results Probl. Cell Differ.,* **17**:85-105, (1991)) may be used. These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. For reviews of such techniques, see Hobbs S. or Murry L.E. (1992) in McGraw Hill Yearbook of Science and Technology, McGraw Hill, New York, N.Y., pp 191-196; or Weissbach and Weissbach (1988) *Methods for Plant Molecular Biology*, Academic Press, New York, N.Y., pp 421-463.

DNKV product may also be expressed in an insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The DNKV product coding sequence may be cloned into a nonessential

region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of DNKV coding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses are then used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which DNKV protein is expressed (Smith *et al., J. Virol.* 46:584, (1983); Engelhard, E.K. *et al., Proc. Nat. Acad. Sci.* **91**:3224-7, (1994)).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, a DNKV product coding sequence may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a nonessential E1 or E3 region of the viral genome will result in a viable virus capable of expressing DNKV protein in infected host cells (Logan and Shenk, *Proc. Natl. Acad. Sci.* **81**:3655-59, (1984). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be required for efficient translation of a DNKV product coding sequence. These signals include the ATG initiation codon and adjacent sequences. In cases where DNKV product coding sequence, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon must be provided. Furthermore, the initiation codon must be in the correct reading frame to ensure transcription of the entire insert. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (Scharf, D. *et al.*, (1994) *Results Probl. Cell Differ.*, **20**:125-62, (1994); Bittner et al., *Methods in Enzymol* **153**:516-544, (1987)).

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., and Battey, I. (1986) Basic Methods in Molecular Biology). Cell-free translation systems can also be employed to produce polypeptides using RNAs derived from the DNA constructs of the present invention.

A host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the protein include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a *"pre-pro"* form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, 293, WI38, etc. have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express DNKV product may be transformed using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler M., *et al.*, *Cell* **11**:223-32, (1977)) and adenine phosphoribosyltransferase (Lowy I., *et al.*, *Cell* **22**:817-23, (1980)) genes which can be employed in *tk-* or *aprt-* cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, *dhfr* which confers resistance to methotrexate (Wigler M., *et al., Proc. Natl. Acad. Sci.* 77:3567-70, (1980)); *npt*, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. *et al., J. Mol. Biol.*, **150**:1-14, (1981)) and *als* or *pat*, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, *supra*). Additional selectable genes have been described, for example, *trpB*, which allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman S.C. and R.C. Mulligan, *Proc. Natl. Acad. Sci.* **85**:8047-51, (1988)). The use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its substrate, GUS, and luciferase and its substrates, luciferin and ATP, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C.A. *et. al., Methods Mol. Biol.*, 55:121-131, (1995)).

Host cells transformed with a nucleotide sequence encoding DNKV product may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The product produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing nucleic acid sequences encoding DNKV product can be designed with signal sequences which direct secretion of DNKV product through a prokaryotic or eukaryotic cell membrane.

The DNKV product may also be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein

purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, Wash.). The inclusion of a protease-cleavable polypeptide linker sequence between the purification domain and DNKV product is useful to facilitate purification. One such expression vector provides for expression of a fusion protein compromising a DNKV polypeptide fused to a polyhistidine region separated by an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography, as described in Porath, *et al.*, *Protein Expression and Purification,* **3**:263-281, (1992)) while the enterokinase cleavage site provides a means for isolating DNKV polypeptide from the fusion protein. *pGEX* vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to ligand-agarose beads (e.g., glutathione-agarose in the case of GST-fusions) followed by elution in the presence of free ligand.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, or other methods, which are well know to those skilled in the art.

The DNKV products can be recovered and purified from recombinant cell cultures by any of a number of methods well known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation

exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

C. Diagnostic applications utilizing nucleic acid sequences

The nucleic acid sequences of the present invention may be used for a variety of diagnostic purposes. The nucleic acid sequences may be used to detect and quantitate expression of the DNKV in patient's cells, e.g. biopsied tissues, by detecting the presence of mRNA coding for DNKV product. Alternatively, the assay may be used to detect soluble DNKV in the serum or blood. This assay typically involves obtaining total mRNA from the tissue or serum and contacting the mRNA with a nucleic acid probe. The probe is a nucleic acid molecule of at least 20 nucleotides, preferably 20-30 nucleotides, capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding DNKV product under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of DNKV. This assay can be used to distinguish between absence, presence, and excess expression of DNKV product and to monitor levels of DNKV expression during therapeutic intervention. In addition, the assay may be used to compare the levels of the DNKV of the invention to the levels of the original kinase sequence from which it has been varied or to levels of other DNKVs, which comparison may have some physiological meaning.

The invention also contemplates the use of the nucleic acid sequences as a diagnostic for diseases resulting from inherited defective DNKV sequences, or diseases in which the ratio of the amount of the original kinase sequence from which the DNKV was varied to the novel DNKVs of the invention is altered. These sequences can be detected by comparing the sequences of the defective

(i.e., mutant) DNKV coding region with that of a normal coding region. Association of the sequence coding for mutant DNKV product with abnormal DNKV product activity may be verified. In addition, sequences encoding mutant DNKV products can be inserted into a suitable vector for expression in a functional assay system (e.g., colorimetric assay, complementation experiments in a variant protein deficient strain of HEK293 cells) as yet another means to verify or identify mutations. Once mutant genes have been identified, one can then screen populations of interest for carriers of the mutant gene.

Individuals carrying mutations in the nucleic acid sequence of the present invention may be detected at the DNA level by a variety of techniques. Nucleic acids used for diagnosis may be obtained from a patient's cells, including but not limited to such as from blood, urine, saliva, placenta, tissue biopsy and autopsy material. Genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki, *et al.*, *Nature* **324**:163-166, (1986)) prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid of the present invention can be used to identify and analyze mutations in the gene of the present invention. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype.

Point mutations can be identified by hybridizing amplified DNA to radiolabeled RNA of the invention or alternatively, radiolabeled antisense DNA sequences of the invention. Sequence changes at specific locations may also be revealed by nuclease protection assays, such RNase and S1 protection or the chemical cleavage method (e.g. Cotton, *et alProc. Natl. Acad. Sci.* USA, **85**:4397-4401, (1985)), or by differences in melting temperatures. *"Molecular beacons"* (Kostrikis L.G. *et al.*, Science **279**:1228-1229, (1998)), hairpin-shaped, single-stranded synthetic oligo- nucleotides containing probe sequences which are complementary to the nucleic acid of the present invention, may also be used to detect point mutations or other sequence changes as well as monitor expression

levels of DNKV product. Such diagnostics would be particularly useful for prenatal testing.

Another method for detecting mutations uses two DNA probes which are designed to hybridize to adjacent regions of a target, with abutting bases, where the region of known or suspected mutation(s) is at or near the abutting bases. The two probes may be joined at the abutting bases, e.g., in the presence of a ligase enzyme, but only if both probes are correctly base paired in the region of probe junction. The presence or absence of mutations is then detectable by the presence or absence of ligated probe.

Also suitable for detecting mutations in the DNKV product coding sequence are oligonucleotide array methods based on sequencing by hybridization (SBH), as described, for example, in U.S. Patent No. 5,547,839. In a typical method, the DNA target analyte is hybridized with an array of oligonucleotides formed on a microchip. The sequence of the target can then be "read" from the pattern of target binding to the array.

D. Gene mapping utilizing nucleic acid sequences

The nucleic acid sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 20-30 bp) from the DNKV cDNA. Computer analysis of the 3' untranslated region is used to rapidly select primers that do not span more than one exon in the genomic DNA, which would complicate the amplification

process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids or using instead radiation hybrids are rapid procedures for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 50 or 60 bases. For a review of this technique, see Verma *et al., Human Chromosomes: a Manual of Basic Techniques*, (1988) Pergamon Press, New York.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in the OMIM database (Center for Medical Genetics, Johns Hopkins University, Baltimore, MD and National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD). The OMIM gene map presents the cytogenetic map location of disease genes and other expressed genes. The OMIM database provides information on diseases associated with the chromosomal location. Such associations include the results of linkage analysis mapped to this interval, and the correlation of translocations and other chromosomal aberrations in this area with the advent of polygenic diseases, such as cancer, in general and prostate cancer in particular.

E. Therapeutic applications of nucleic acid sequences

Nucleic acid sequences of the invention may also be used for therapeutic purposes. Turning first to the second aspect of the invention (i.e. inhibition of expression of DNKV), expression of DNKV product may be modulated through antisense technology, which controls gene expression through hybridization of complementary nucleic acid sequences, i.e. antisense DNA or RNA, to the control, 5' or coding regions of the gene encoding DNKV product. For example, the 5' coding portion of the nucleic acid sequence which codes for the product of the present invention is used to design an antisense oligonucleotide of from about 10 to 40 base pairs in length. Oligonucleotides derived from the transcription start site, e.g. between positions -10 and +10 from the start site, are preferred. An antisense DNA oligonucleotide is designed to be complementary to a region of the nucleic acid sequence involved in transcription (Lee *et al., Nucl. Acids, Res.*, **6**:3073, (1979); Cooney et al., Science **241**:456, (1988); and Dervan *et al., Science* **251**:1360, (1991)), thereby preventing transcription and the production of the DNKV products. An antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into the DNKV products (Okano *J. Neurochem.* **56**:560, (1991)). The antisense constructs can be delivered to cells by procedures known in the art such that the antisense RNA or DNA may be expressed *in vivo*. The antisense may be antisense mRNA or DNA sequence capable of coding such antisense mRNA. The antisense mRNA or the DNA coding thereof can be complementary to the full sequence of nucleic acid sequences coding for the DNKV protein or to a fragment of such a sequence which is sufficient to inhibit production of a protein product.

Turning now to the first aspect of the invention, i.e. expression of DNKV, expression of DNKV product may be increased by providing coding sequences for coding for said product under the control of suitable control elements ending its expression in the desired host.

The nucleic acid sequences of the invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise

a therapeutically effective amount of the compound, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The products of the invention as well as any activators and deactivators compounds (see below) which are polypeptides, may also be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as *"gene therapy."* Cells from a patient may be engineered with a nucleic acid sequence (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered in vivo for expression of a polypeptide *in vivo* by procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a product of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells *in vivo* after combination with a suitable delivery vehicle.

Retroviruses from which the retroviral plasmid vectors mentioned above may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be

transfected include, but are not limited to, the *PE501*, *PA317*, *psi-2*, *psi-AM*, *PA12*, *T19-14X*, *VT-19-17-H2*, *psi-CRE*, *psi-CRIP*, *GP+E-86*, *GP+envAm12*, and *DAN* cell lines as described in Miller (*Human Gene Therapy*, Vol. **1**, pg. 5-14, (1990)). The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

The genes introduced into cells may be placed under the control of inducible promoters, such as the radiation-inducible Egr-1 promoter, (Maceri, H.J., *et al., Cancer Res.*, **56**(19):4311 (1996)), to stimulate DNKV production or antisense inhibition in response to radiation, e.g., radiation therapy for treating tumors.

Example II. DNKV product

The substantially purified DNKV product of the invention has been defined above as the product coded from the nucleic acid sequence of the invention. Preferably the amino acid sequence is an amino acid sequence having at least 90% identity to any one of the sequences coded by the nucleic acid sequence of SEQ ID NO: 1 to SEQ ID NO: 91 provided that the amino acid sequence is not identical to that of the original sequence from which it has been varied. The protein or polypeptide may be in mature and/or modified form, also

as defined above. Also contemplated are protein fragments having at least 10 contiguous amino acid residues, preferably at least 10-20 residues, derived from the DNKV product, as well as homologues as explained above.

The sequence variations are preferably those that are considered conserved substitutions, as defined above. Thus, for example, a protein with a sequence having at least 90% sequence identity with any of the products depicted by SEQ ID NO: 92 to SEQ ID NO: 182, preferably by utilizing conserved substitutions as defined above is also part of the invention, and provided that it is not identical to the original peptide from which it has been varied. The DNKV product may be (i) one in which one or more of the amino acid residues in a sequence listed above are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue), or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the DNKV product is fused with another compound, such as a compound to increase the half-life of the protein (for example, polyethylene glycol (PEG)), or a moiety which serves as targeting means to direct the protein to its target tissue or target cell population (such as an antibody), or (iv) one in which additional amino acids are fused to the DNKV product. Such fragments, DNKVS and derivatives are deemed to be within the scope of those skilled in the art from the teachings herein.

A. Preparation of DNKV product

Recombinant methods for producing and isolating the DNKV product, and fragments of the protein are described above.

In addition to recombinant production, fragments and portions of DNKV product may be produced by direct peptide synthesis using solid-phase techniques (cf. Stewart *et al.*, (1969) Solid-Phase Peptide Synthesis, WH Freeman Co, San Francisco; Merrifield J., *J. Am. Chem. Soc.*, **85**:2149-2154, (1963)). In vitro peptide synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems

431A Peptide Synthesizer (Perkin Elmer, Foster City, Calif.) in accordance with the instructions provided by the manufacturer. Fragments of DNKV product may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

B. Therapeutic uses and compositions utilizing the DNKV product

The DNKV product of the invention is generally useful in treating diseases and disorders which are characterized by a lower than normal level of DNKV expression, and or diseases which can be cured or ameliorated by raising the level of the DNKV product, even if the level is normal.

DNKV products or fragments may be administered by any of a number of routes and methods designed to provide a consistent and predictable concentration of compound at the target organ or tissue. The product-containing compositions may be administered alone or in combination with other agents, such as stabilizing compounds, and/or in combination with other pharmaceutical agents such as drugs or hormones.

DNKV product-containing compositions may be administered by a number of routes including, but not limited to oral, intravenous, intramuscular, transdermal, subcutaneous, topical, sublingual, or rectal means as well as by nasal application. DNKV product-containing compositions may also be administered via liposomes. Such administration routes and appropriate formulations are generally known to those of skill in the art.

The product can be given via intravenous or intraperitoneal injection. Similarly, the product may be injected to other localized regions of the body. The product may also be administered via nasal insufflation. Enteral administration is also possible. For such administration, the product should be formulated into an appropriate capsule or elixir for oral administration, or into a suppository for rectal administration.

The foregoing exemplary administration modes will likely require that the product be formulated into an appropriate carrier, including ointments, gels,

suppositories. Appropriate formulations are well known to persons skilled in the art.

Dosage of the product will vary, depending upon the potency and therapeutic index of the particular polypeptide selected.

A therapeutic composition for use in the treatment method can include the product in a sterile injectable solution, the polypeptide in an oral delivery vehicle, the product in an aerosol suitable for nasal administration, or the product in a nebulized form, all prepared according to well known methods. Such compositions comprise a therapeutically effective amount of the compound, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof.

Example III. Screening methods for activators and deactivators (inhibitors)

The present invention also includes an assay for identifying molecules, such as synthetic drugs, antibodies, peptides, or other molecules, which have a modulating effect on the activity of the DNKV product, e.g. activators or deactivators of the DNKV product of the present invention. Such an assay comprises the steps of providing an DNKV product encoded by the nucleic acid sequences of the present invention, contacting the DNKV protein with one or more candidate molecules to determine the candidate molecules modulating effect on the activity of the DNKV product, and selecting from the molecules a candidate's molecule capable of modulating DNKV product physiological activity.

The DNKV product, its catalytic or immunogenic fragments or oligopeptides thereof, can be used for screening therapeutic compounds in any of a variety of drug screening techniques. The fragment employed in such a test may be free in solution, affixed to a solid support, borne on a cell membrane or located intracellularly. The formation of binding complexes, between DNKV

product and the agent being tested, may be measured. Alternatively, the activator or deactivator may work by serving as agonist or antagonist, respectively, of the DNKV receptor, binding entity or target site, and their effect may be determined in connection with any of the above.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the DNKV product is described in detail by Geysen in PCT Application WO 84/03564, published on Sep. 13, 1984. In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with the full DNKV product or with fragments of DNKV product and washed. Bound DNKV product is then detected by methods well known in the art. Substantially purified DNKV product can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

Antibodies to the DNKV product, as described in Example IV below, may also be used in screening assays according to methods well known in the art. For example, a *"sandwich"* assay may be performed, in which an anti-DNKV antibody is affixed to a solid surface such as a microtiter plate and DNKV product is added. Such an assay can be used to capture compounds which bind to the variant DNKV. Alternatively, such an assay may be used to measure the ability of compounds to influence with the binding of variant product to the variant receptor, and then select those compounds which effect the binding.

Example IV. Anti-DNKV antibodies/distinguishing antibodies

A. Synthesis

In still another aspect of the invention, the purified DNKV product is used to produce anti-DNKV antibodies which have diagnostic and therapeutic uses related to the activity, distribution, and expression of the DNKV product. As

indicated above, the antibodies may also be directed solely to amino acid sequences present in the variant but not present in the original kinase sequence, or to sequences present only in the original kinase sequence but not in the DNKV (distinguishing antibodies).

Antibodies to the DNKV product or to the distinguishing sequence present only in the DNKV or only in the original kinase sequence (the latter termed *"distinguishing antibodies"*) may be generated by methods well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, humanized, single chain, Fab fragments and fragments produced by an Fab expression library. Antibodies, i.e., those which inhibit dimer formation, are especially preferred for therapeutic use.

A fragment of the DNKV product for antibody induction does not require biological activity but have to feature immunological activity; however, the protein fragment or oligopeptide must be antigenic. Peptides used to induce specific antibodies may have an amino acid sequence consisting of at least five amino acids, preferably at least 10 amino acids of any sequences coded by the nucleic acid sequence of SEQ ID NO: 1 to SEQ ID NO: 91 or in distinguishing sequences present only in the DNKV or only in the original kinase sequence as explained above. Preferably they should mimic a portion of the amino acid sequence of the natural protein and may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of DNKV protein amino acids may be fused with those of another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule. Procedures well known in the art can be used for the production of antibodies to DNKV product.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, etc may be immunized by injection with DNKV product or any portion, fragment or oligopeptide which retains immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include but are not limited to Freund's,

mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (bacilli Calmette-Guerin) and *Corynebacterium parvum* are potentially useful human adjuvants.

Monoclonal antibodies to DNKV protein may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Koehler and Milstein (*Nature* **256**:495-497, (1975)), the human B-cell hybridoma technique (Kosbor *et al., Immunol. Today* **4**:72, (1983); Cote *et al., Proc. Natl. Acad. Sci.* **80**:2026-2030, (1983)) and the EBV-hybridoma technique (Cole, *et al., Mol. Cell Biol.* 62:109-120, (1984)).

Techniques developed for the production of *"chimeric antibodies"*, the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can also be used (Morrison *et al., Proc. Natl. Acad. Sci.* **81**:6851-6855, (1984); Neuberger *et al., Nature* **312**:604-608, (1984); Takeda *et al., Nature* **314**:452-454, (1985)). Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce single-chain antibodies specific for the DNKV protein.

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi *et al.* (*Proc. Natl. Acad. Sci.* **86**:3833-3837, 1989)), and Winter G and Milstein C., (*Nature* **349**:293-299, (1991)).

Antibody fragments which contain specific binding sites for DNKV protein may also be generated. For example, such fragments include, but are not limited to, the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal

Fab fragments with the desired specificity (Huse W.D. *et al., Science* 256:1275-1281, (1989)).

B. Diagnostic applications of antibodies

A variety of protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the formation of complexes between the DNKV product and its specific antibody and the measurement of complex formation. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two noninterfering epitopes on a specific DNKV product is preferred, but a competitive binding assay may also be employed. These assays are described in Maddox D.E., *et al.,* (*J. Exp. Med.* **158**:1211, (1983)).

Antibodies which specifically bind DNKV product or distinguishing antibodies which bind to sequences which distinguish the DNKV from the original kinase sequence (as explained above) are useful for the diagnosis of conditions or diseases characterized by expression of the novel DNKV of the invention (where normally it is not expressed) by over or under expression of DNKV as well as for detection of diseases in which the proportion between the amount of the DNKV of the invention and the original kinase sequence from which it varied is altered. Alternatively, such antibodies may be used in assays to monitor patients being treated with DNKV product, its activators, or its deactivators. Diagnostic assays for DNKV protein include methods utilizing the antibody and a label to detect DNKV product in human body fluids or extracts of cells or tissues. The products and antibodies of the present invention may be used with or without modification. Frequently, the proteins and antibodies will be labeled by joining them, either covalently or noncovalently, with a reporter molecule. A wide variety of reporter molecules are known in the art.

A variety of protocols for measuring the DNKV product, using either polyclonal or monoclonal antibodies specific for the respective protein are known

in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescent activated cell sorting (FACS). As noted above, a two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on DNKV product is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, *et al. (supra)*. Such protocols provide a basis for diagnosing altered or abnormal levels of DNKV product expression. Normal or standard values for DNKV product expression are established by combining body fluids or cell extracts taken from normal subjects, preferably human, with antibody to DNKV product under conditions suitable for complex formation which are well known in the art. The amount of standard complex formation may be quantified by various methods, preferably by photometric methods. Then, standard values obtained from normal samples may be compared with values obtained from samples from subjects potentially affected by disease. Deviation between standard and subject values establishes the presence of disease state.

The antibody assays are useful to determine the level of DNKV product present in a body fluid sample, in order to determine whether it is being expressed at all, whether it is being overexpressed or underexpressed in the tissue, or as an indication of how DNKV levels of variable products are responding to drug treatment.

C. Therapeutic uses of antibodies

In addition to their diagnostic use the antibodies may have a therapeutical utility in blocking or decreasing the activity of the DNKV product in pathological conditions where beneficial effect can be achieved by such a decrease. Again, distinguishing antibodies may be used to neutralize differentially either the DNKV or the original kinase sequence as the case may be.

The antibody employed is preferably a humanized monoclonal antibody, or a human Mab produced by known globulin-gene library methods. The

antibody is administered typically as a sterile solution by IV injection, although other parenteral routes may be suitable. Typically, the antibody is administered in an amount between about 1-15 mg/kg body weight of the subject. Treatment is continued, e.g., with dosing every 1-7 days, until a therapeutic improvement is seen.

Although the invention has been described with reference to specific methods and embodiments, it is appreciated that various modifications and changes may be made without departing from the invention.